EXPOSURE, SENSITIZATION AND ALLERGY TO INDUSTRIAL ENZYMES

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To Sanna-Leena,
Ilkka, Sini and Jukka

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SUMMARY

The production and use of industrial enzymes have increased markedly during the last few decades. Today, enzymes are used, for example, in the detergent, food, feed, textile and pulp and paper industries. Respiratory allergies to powdered microbial enzymes surfaced in the late 1960s in the detergent industry. With improvements in industrial hygiene, the problem abated. Since the 1980s, allergies have emerged in other industries however, notably in bakeries.

A series of studies on enzyme allergy was performed in 1992–1997. The aim was to assess exposure and allergy to enzymes in Finnish enzyme manufacturing and industries using enzymes. Investigations were performed in four bakeries, one flour mill, one rye crisp factory, one detergent factory, four animal feed factories, one biotechnical research laboratory and one biotechnical plant having both research and production units.

For determining α-amylase, a catalytic method was used which detects also the inherent amylase of flour. For protease detection both a catalytic method and a more specific immunologic procedure were used. Cellulase and xylanase were measured with an immunologic method.

Powdered enzyme-containing additives were used in the bakeries, where high α-amylase levels, up to 6.6 μg/m^3, were found during dough making. In other locations, the levels were generally lower, below 0.2 μg/m^3. In addition, xylanase concentrations of 2–200 ng/m^3 (mean 65 ng/m^3) were found, possibly also due to inherent xylanase. Enzyme-containing additives were mixed in the four mill, and α-amylase concentrations up to 1.1 μg/m^3 and cellulase concentrations up to 180 ng/m^3 were determined at the mixing sites. In the rye crisp factory the α-amylase levels were lower than in the bakeries (mean value 0.1 μg/m^3 for personal samples and 0.03 μg/m^3 for stationary samples). The cellulase concentrations ranged from 25 to 160 ng/m^3 in different phases of the mixing, dough making and bread forming. At the same sites, lower levels (7–40 ng/m^3) of xylanase were measured.

In the animal feed factories, the nonspecific assay showed high levels of protease (up to 0.4–2.9 μg/m^3) and α-amylase (up to 0.2 μg/m^3), which coincided with the high total dust levels but not with the amount of added enzyme.

In the detergent factory, the protease levels, measured with a catalytic method, were generally below 50 ng/m^3, but at the enzyme mixing site very high concentrations, above 1000 ng/m^3, were found. The analysis with an immunologic method gave results of the same

order, indicating that the main origin of the protease was the added enzyme.

Few measurements prior this study from the enzyme manufacturing industry had indicated cellulase concentrations on the order of 50 ng/m^3 in laboratory work. Judging from job descriptions, much higher enzyme concentrations probably occurred occasionally during the mixing, drying and packing of enzymes.

The prevalence of sensitization to enzymes, assessed by skin prick testing, was 7.8% in the bakeries, 4.8% in the flour mill and 2.7% in the rye crisp factory. When the office personnel was excluded, the figures were 8.4%, 5.3% and 3%, respectively. In the animal feed industry the corresponding prevalences were 4.6% and 7.1%, and in the detergent industry 11.8% and 22.5%. In the biotechnical research laboratory 11.7% of the workers and in the biotechnical plant 12.6% of the workers were sensitized. In the category of research, laboratory and enzyme manufacturing work, the rates were 12.6% and 15.4%, respectively. A statistically significant exposure-response linear trend was demonstrated among the biotechnical workers. Atopy, as demonstrated using skin prick testing, increased the risk of sensitization three to five times among the workers studied, except in the detergent factory.

Sensitization to enzymes was associated with work-related respiratory symptoms in all the industries studied. Several cases of specific occupational asthma or rhinitis due to enzymes were diagnosed later, thus verifying the causal connection of sensitization to clinical allergy. The bronchial challenge method used proved to be practical for challenges with powdered enzymes.

Sensitization was found for previously well-known enzymes, such as protease in the detergent industry and α-amylase in the bakeries. Lipase and cellulase were also shown to be allergens in the detergent industry. In addition, it was found that phytase causes sensitization in enzyme production and the animal feed industry. Sensitization to cellulase and xylanase was common due to the increasing manufacture of these enzymes in Finland. Immunoblotting showed that the antigens of α-amylases of bacterial and fungal origin differed from each other, as the sera from persons sensitized to fungal amylase did not bind to bacterial amylase, and vice versa.

Development and international standardization are urgently needed to establish methods for measuring air concentrations of enzymes. For the prevention of sensitization to enzymes and allergic diseases caused by them, the risk of allergy has to be recognized at workplaces, and exposure to enzymes must be kept to a minimum.

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ABBREVIATIONS

ELISA	enzyme-linked immunosorbent assay
FIOH	Finnish Institute of Occupational Health
$FEV_{1.0}$	forced expiratory volume in 1 second
IgE	immunoglobulin E
kDa	kilo Dalton
MW	molecular weight
OA	occupational asthma
OEL	occupational exposure limit
PEFR	peak expiratory flow rate
py	person-year
RAST	radioallergosorbent test
TLV	threshold limit value
SPT	skin prick test
wt/vol	weight/volume

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by the Roman numerals indicated below:

I Vanhanen M, Tuomi T, Hokkanen H, Tupasela O, Tuomainen A, Holmberg PC, Leisola M, Nordman H. Enzyme exposure and enzyme sensitization in the baking industry.
Occup Environ Med 1996;53:670–676.

II Vanhanen M, Tuomi T, Nordman H, Tupasela O, Holmberg PC, Miettinen M, Mutanen P, Leisola M. Sensitization to industrial enzymes in enzyme research and production.
Scand J Work Environ Health 1997;23:385–391.

III Vanhanen M, Tuomi T, Tiikkainen U, Tupasela O, Voutilainen R, Nordman H. Risk of enzyme allergy in the detergent industry.
Occup Environ Med 2000;57:121–125.

IV Vanhanen M, Tuomi T, Tiikkainen U, Tupasela O, Tuomainen A, Luukkonen R, Nordman H. Sensitisation to enzymes in the animal feed industry.
Occup Environ Med 2001;58:119–123.

V Vanhanen M, Tuomi T, Tupasela O, Keskinen H, Tuppurainen M, Hytönen M, Tarvainen K, Kanerva L, Nordman H. Cellulase allergy and challenge tests with cellulase using immunologic assessment.
Scand J Work Environ Health 2000; 26:250–256.

1. INTRODUCTION

Enzymes are proteins that are present in all cells and that catalyze chemical reactions. Along with the progress of modern biotechnology during the past 20 years, the production and use of industrial enzymes have multiplied. For example, by breaking down protein, carbohydrate and lipid molecules in stains, enzymes enhance the action of detergents. The baking process is improved by different enzyme actions on the dough, and the stone washing effect of jeans is achieved by cellulotic enzyme action on the fabric.

The allergenic potency of enzymes was confronted in the enzyme production and detergent industries worldwide in the late 1960s. The health effects were primarily respiratory allergies (asthma, rhinitis). As a consequence of allergies in the detergent industry, major industrial hygiene improvements were made, such as encapsulation of the enzyme product and other means of decreasing exposures. As a result, there has been a great reduction in the occurrence of allergies in the detergent industry since the mid-1970s. However, when enzymes were introduced gradually to other industries, allergies emerged in, for example, the pharmaceutical and baking industries in the 1980s.

In Finland, the experience with enzyme-induced allergies started to grow in the beginning of the 1990s when allergies emerged in the expanding enzyme manufacturing industry. A research project was started at the Finnish Institute of Occupational Health (FIOH) with the aim of gathering data on the use of enzymes in Finland and assessing exposure, sensitization and allergic symptoms due to enzymes.

2. REVIEW OF THE LITERATURE

2.1. What enzymes are

Enzymes are proteins that are present in all living cells, where they perform a vital function by controlling the metabolic processes whereby nutrients are converted into energy and fresh cell material. Enzymes perform these tasks as catalysts; in other words, they speed up the chemical processes, without being consumed in the process (Stryer 1999). A unique feature of enzymes is also their great specificity (i.e., each enzyme can break down or synthesize one particular compound or work on a specific bond only). Enzymes are also very efficient, one enzyme molecule being able to catalyze the breakdown of millions of molecules. These features are utilized in industrial processes. Furthermore, being proteins, they are readily degradable and, as such, are ideal for the environment. Being formed to work in living cells, enzymes can work at atmospheric pressure and under mild conditions in terms of temperature and acidity (pH). Most enzymes function optimally at a temperature of 30–70°C and at pH values that are near neutral (pH 7).

2.2. History of enzyme use and technology

Enzymes have been used by humans throughout the ages, either in the form of vegetables rich in enzymes or in the form of microorganisms used for a variety of purposes, for instance, brewing processes, baking, cheese manufacturing and the production of alcohol. In 1876, William Kühne proposed the term “enzyme”, which means “in yeast” and is derived from the Greek words *“en”* and *“zyme”* (Voet & Voet 1995). Development in protein chemistry methods in the 19th century, and in the beginning of the 20th century, led to the extraction and production of enzymes from animal and plant tissue, such as rennet from calves’ stomachs (for cheese production) and pancreatic extracts for bating in leather manufacturing and for use in detergents (Gerhartz 1990).

The development of the submerged-culture technique represented a major advance in enzyme technology since it permitted the large-scale production of microorganisms for industrial purposes. Such a technique was introduced early in the 1950s at a time when the production of bacterial amylases was begun for the textile industry by a Danish company. Very soon other microbial enzymes were also produced. In 1959, the first detergent containing a bacterial protease

was introduced. The manufacture of enzymes for industrial purposes progressed rapidly after 1965, due mainly to the increasing use of enzymes in detergents.

2.3. Modern production of enzymes by microbes

Initial laboratory work includes the selection and modification of microorganisms so that they are capable of producing the desired enzymes at high yields (Gerhartz 1990). The selected strains are combined with specially selected sterile nutrient media in a "seed tank", where the biological amplification occurs. Once sufficient mass has accumulated, the culture is aseptically transferred to a large fermentation tank. During the ensuing fermentation, enzyme production occurs. Enzymes are then separated from the biomass through a series of filtration steps. The enzyme slurry is pumped to the filter system where a major portion of the suspended solids is separated from the enzyme liquid. The enzyme liquid is concentrated with an evaporator and refiltered to remove unwanted bacterial contamination. Following filtration, enzyme activity is stabilized, and preservative materials are added to the product. The final commercial product is either in liquid, powder or granulated (encapsulated) form. The latter two forms are produced using spray-drying procedures.

Usually the commercial enzyme product does not need to be "pure" in order to perform the task for which it is intended. Thus it may contain other enzyme activities produced by the microorganism, and other proteins or parts of proteins from the media as well.

Currently, the most common microbes used in the production of enzymes are the molds *Aspergillus oryzae*, *A.niger* and *Trichoderma reesei* and the bacteria *Bacillus subtilis* and *Bacillus amyloliquefaciens*. With the tools of genetic engineering, the primary gene coding the enzyme may come from a separate microbe (or from, e.g., any mammalian cell) rather than from the host microbe.

2.4. Classification of enzymes

According to the reactions they catalyze, enzymes can be classified into oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Stryer 1999). In industrial use, by far the most important group is the hydrolases. Hydrolases cleave certain bondages of molecules hydrolytically. They are separated and named

according to the substances they cleave (e.g., amylases, cellulases, hemicellulases, proteases, pectinases, lipases and lactases).

2.5. Applications of industrial enzymes

The detergent and food industries are the most important users of enzymes (Gerhartz 1990, AMFEP 2001). However, their application is increasing, for example, in the textile and animal feed industries.

In the *detergent industry* the most common enzymes are *Bacillus*-derived proteases. Several different proteases are available, with differences, for example, in the pH and temperature range in which they function. Other enzymes, such as α-amylases, lipases, and cellulases, have been introduced later. In addition to the traditional use of enzymes in laundry detergents, enzymes have recently been incorporated into dishwashing detergents. The detergent is usually less than 1% enzymes. Since the early 1970s the detergent enzymes have been granulated or encapsulated products. The role of enzymes is to break down protein, lipid and carbohydrate molecules of stains in fabrics. Enzymes are used also in *personal care products*, for example, in contact lense cleansing solutions and toothpastes.

In *bakeries* enzymes have been used increasingly since the early 1980s; now most (80–90%) Finnish bakeries use enzyme-containing additives, also called "bread improvers". The enzyme usually comprises only 0.2–1% of the total weight of the additive. The amount of the additive in the dough is about 1%. α-Amylase of fungal origin (*A. oryzae*) is by far the most common enzyme; others are α-amylase of bacterial origin, glucoamylase, xylanase, lipase and glucose oxidase. Although liquid and paste forms have been available for several years, powdered products are still the most commonly used. The benefits of enzymes in baking are the improved dough handling properties, the increased bread volume, the improved crumb structure and the retarded staling process (Poutanen 1997).

α-Amylase is used to speed up the degradation of starch in the *production of sugar*. Glucose isomerase converts glucose into fructose and is utilized in the production of "high fructose syrup", used in sweetening of foodstuffs.

In the *alcohol and brewing industries*, enzymes are used to break down starch into smaller molecules that the yeast can transform into alcohol. Traditionally, enzymes have been provided by adding malt. Because of their effectiveness, standardized activity and easier handling, modern enzymes have largely replaced malt. Enzymes improve also the filtering process. α-Amylase, glucoamylase, cellulololytic enzymes and proteases are used. Cellulases and

pectinases are also used to in the production of *fruit juice*. Applications of enzyme use have been developed even for winemaking.

In *cheese manufacturing*, the traditional enzyme, calf rennet, is being replaced by microbial chymosin. Lactase is used to cleavage lactose in dairy products. Other applications of enzymes in food industry are being developed, e.g. in vegetable oil production and the food functionality industry.

In *pharmaceutical industry*, several enzymes are used as constituents of medicines (e.g. digestive aids) or as preservatives.

When included in *animal feed,* enzymes improve the digestion of the feed, especially in monogastric animals such as poultry and pigs. The enzymes break down digestible proteins and starch from the feed fibers. In addition, enzymes can be used to increase the availability of minerals, especially phosphorus, from the feed. Better degradation of feed also makes the excrements more solid. Consequently, enzymes are marketed also as having a favorable environmental impact.

Applications of enzymes in the *textile industry* are expanding rapidly. For example, denim is given the “stone-washing” effect, and the fuzz can be removed from clothes by the action of cellulase (Tenkanen et al 1999).

In *the pulp and paper industry,* xylanases are used to help bleach the pulp and thus decrease the need for chlorine compounds. Cellulase can be used for the de-inking of waste paper, and lipases are used to reduce pitch deposits in paper mills (Viikari et al 1998).

In the *leather industry*, extra proteins and fats can be removed from the hides by using microbial proteases and lipases, in addition to the traditional pancreatic protease.

The most common enzymes used in different industries and the estimated number of exposed employees in Finland are listed in Table 1.

Table 1. Common industrial enzymes, their applications and estimated number of exposed workers in Finland

Application	Enzyme	Product form	Exposed employees in Finland (estimated)*
Alcohol production	α-amylase, amyloglucosidase, cellulase	liquid	some 10's
Animal feed	α-amylase, amyloglucosidase, cellulase, xylanase, protease, phytase	powder, granule, liquid	about 100
Baking	α-amylase, amyloglucosidase, cellulase, xylanase, glucose oxidase, protease	powder (paste and liquid products taken into use in the late 1990s)	3000–4000
Brewing	α-amylase, amyloglucosidase, cellulase, protease	liquid	some 10's
Cheese making	chymosin	liquid	some 10's
Detergent industry	protease, lipase, cellulase, amylase	granules, liquid	some 10's
Leather industry	protease, lipase	powder, liquid	some 10's
Pulp and paper industry	α-amylase, cellulase, xylanase	liquid	some 10's
Starch and sugar industry	α-amylase, amyloglucosidase, glucose isomerase	liquid	some 10's
Textile industry	α-amylase, cellulase	liquid, powder	some 10's
Enzyme production	tens of enzymes	liquid, powder, granules	200–300

* In estimation of the amount of employees, data from the manufacturing statistics and interviews of representatives of the industry were used.

2.6. Health effects of industrial enzymes

2.6.1. Respiratory allergies caused by enzymes

Reviews on allergies from enzymes have also been published recently (Brisman 1994, Houba et al 1998a, Bernstein 1999a).

Studies on respiratory allergies caused by enzymes are summarized in Tables 2–4 according to industry.

2.6.1.1. Detergent industry

A marked enzyme allergy problem appeared in the late 1960s and early 1970s, when clusters of enzyme allergy emerged rapidly in enzyme production and the detergent industries. The appearence was linked to the expanded production of *B. subtilis* proteases. The first report was published by Flindt (1969), who described asthmatic symptoms emerging in a detergent factory during the course of the first year that proteases were introduced in the plant. Out of a group of symptomatic workers, 25 had positive skin prick tests (SPTs) to one or two protease products (Alcalase®, Maxatase®). After this report, epidemiological studies started to accumulate from the industry. The sensitization rate was 5–50%, and 5–30% had work-related symptoms (Wüthrich & Ott 1969, Greenberg et al 1970, McMurrain 1970, Newhouse et al 1970, Shapiro et al 1971, Weill et al 1971, Göthe et al 1972, Gilson et al 1976, Belin & Norman 1977, Juniper et al 1977, Zachariae 1981, Juniper & Roberts 1984, Pepys et al 1985, Flood et al 1985). The symptoms were primarily respiratory (asthma, rhinitis), and only a few skin symptoms were reported, whose origin was considered to be irritation, not sensitization.

After the initial reports of high allergy prevalences in the industry, the rapid growth of enzyme detergents was temporarily set back in the early 1970s. Vigorous actions were taken to solve the problem, including the development of encapsulated enzyme products (to prevent dusting) and improvements in industrial hygiene at the worksites, such as enclosure of processes and use of respiratory protective equipment. Some of the factories ceased using enzymes. Some adopted the practice of excluding atopics from enzyme work (Newhouse et al 1970, Witmeur et al 1973, Juniper et al 1977). A major reduction in sensitization and symptoms was reported among employees (Gilson et al 1976, Juniper et al 1977, Juniper & Roberts 1984, Pepys et al 1985, Flood et al 1985). The enzyme allergy problem in the detergent industry seemed to have abated. Large multinational companies reported a yearly incidence of 2–3% new cases of

sensitization and a prevalence of up to 10% but few or no cases of asthma during the 1990s (Gaines 1994, Cathcart et al 1997, Sarlo et al 1997a, Schweigert et al 2000). Recently, however, a high prevalence of sensitization to enzymes (26%) and a prevalence of 16% for work-related lower-respiratory symptoms accompanied with sensitization were reported in a detergent factory in the United Kingdom (Cullinan et al 2000).

In Finland, little data exist on allergies in the detergent industry. A case report described two employees, a processman and a packer, who probably had enzyme-induced asthma. Their symptoms started in 1967, about one year after the introduction of enzymes in the factory, and sentitization to the protease used was proved by scratch tests in 1969 (Stubb 1972).

2.6.1.2. Pharmaceutical industry, health care and related occupations

Several case reports and surveys in small populations of, for example, food technologists and pharmaceutical workers with respiratory symptoms and sensitization to plant-derived papain were published in the 1970s and 1980s (Milne & Brand 1975, Flindt 1978, Flindt 1979, Baur & Fruhmann 1979a, Baur et al 1982, Novey et al 1980). Allergies due to chymotrypsin and trypsin were reported by Howe et al (1961) and Zweiman et al (1967), and due to pancreatic extracts by Wiessmann and Baur (1985) and by Hayes and Newman Taylor (1991). Asthma due to pepsin in pharmaceutical employees was described by Maisel (1940) and Cartier et al (1984) and to pectinase by Hartmann et al (1983). Galleguillos and Rodriquez (1978) and Baur and Fruhmann (1979b) reported asthma due to bromelain. In the 1990s, high prevalences of sensitization to α-amylase and lactase were reported in the pharmaceutical industry (Losada et al 1992, Muir et al 1997, Bernstein et al 1999b). A detergent protease, subtilisin, caused asthma in a hospital worker who cleaned instruments (Lemiere 1996). The first report of cellulase as an occupational allergen was that by Ransom and Schuster (1981): the enzyme caused astma in a laboratory worker during plant cloning experiments.

In Finland, papain caused sensitization and rhinitis or asthma in three laboratory employees in a laboratory that used papain as a substrate in vaccine production in 1984 and in one laboratory employee in 1994 (Finnish Register of Occupational Diseases). A case of papain allergy in a cosmetologist was reported in 1993 (Niinimäki et al 1993).

2.6.1.3. Baking industry

The first report of *Aspergillus*-derived α-amylase allergy was published by Flindt in 1979, when five out of eight symptomatic employees in an enzyme-handling factory were sensitized to α-amylase. In the mid-1980s, reports from allergies induced by exposure to α-amylase in the baking industry started to appear. Baur et al (1986) reported sensitization, in a radioallergosorbent test (RAST), to α-amylase in 34% of 27 symptomatic bakery workers in Germany. In a subsequent paper, Baur et al (1988) reported a sensitization rate (by RAST) of 24% for α-amylase, 8% for hemicellulase or cellulase, and 5% for amyloglucosidase. In Sweden, Brisman and Belin (1991) published a report on four symptomatic workers in a factory where amylase-contained baking additives were prepared. In Spain, Quirce et al (1992) described five symptomatic bakers. In Italy, 17 (7.5%) of 226 bakers and pastry makers were sensitized to enzymes (De Zotti et al 1994). In the United Kingdom, 5% of 344 subjects were sensitized in bakery or flour mill work (Cullinan et al 1994), and up to 16% sensitization was reported in a selected plant bakery population (Smith et al 1997). In The Netherlands, 9% of 178 bakery workers were sensitized to α-amylase (Houba et al 1996). A German study comprising a retrospective analysis of sera from 171 symptomatic bakers revealed a sensitization rate of 23% for α-amylase, 8% for amyloglucosidase, 13% for cellulase and 11% for xylanase (Sander et al 1998). In Scotland, 15% of 205 bakery employees were found to be sensitized to α-amylase by RAST (Jeffrey et al 1999). In the United Kingdom, 5% of 264 employees were sensitized to amylase (Nieuwenhuijsen et al 1999).

Few longitudinal studies have been published on the incidence of enzyme allergy in the baking industry. In a cohort of Italian trainee bakers, 125 subjects were tested at 6, 18 and 30 months after the baseline examination. At the baseline, 4 were sensitized to flour or α-amylase; at 30 months, the corresponding number was 10 sensitized to flours, 3 of whom also showed sensitization to amylase (De Zotti & Bovenzi 2000). In the United Kingdom, a nested case-control analysis of a cohort of new bakers was reported recently (Cullinan et al 2001). Out of 300 bakers, 21 had developed sensitization to flour, 2.2. cases per 100 person-years (py), and 24 to α-amylase, 2.5 cases per 100 py.

A correlation between α-amylase and flour sensitization was found in studies in which both substances were assessed. For example, the amylase/flour sensitization prevalences were 5%/5% (Cullinan et al 1994), 7.5%/11.9% (De Zotti et al 1994), 9%/8% (Houba et al 1996), 19%/16% (Baur et al 1998a); 16%/6% (Smith & Smith 1998), and 15%/

24% (Jeffrey et al 1999). Co-sensitization (amylase and flour) was common.

The reported work-related respiratory symptoms in bakeries have a wide range: from a prevalence of 0.5% for asthmatic symptoms and 2.6% for rhinitis (Smith & Smith 1998) to 33% for rhinitis and dyspnea (Baur et al 1998a). A high prevalence of asthmatic symptoms (20.9%) was reported in small bakeries in Scotland (Jeffrey et al 1999). In a United Kingdom cohort of new bakers, the incidence was 11.8/100 py for work-related eye or nose symptoms and 4.1/100 py for chest symptoms (Cullinan et al 2001). The incidence of work-related chest symptoms in the presence of a positive SPT to flour or amylase was 1/100 py.

In Finland, only a few bakery workers, out of a total of about 9000 people per year working in the industry, have been diagnosed as having occupational disease as a result of exposure to enzymes. In 1990–1999, altogether 263 cases of occupational asthma due to flour exposure and only 3 due to amylase exposure were reported, as were 278 cases of rhinitis due to flour exposure, and 3 cases due to amylase exposure (Finnish Register of Occupational Diseases). The following reasons have been proposed: (1) flour-induced allergy is primarily searched for and diagnosed, leaving simultaneous enzyme allergy unrecorded, and (2) workers and health professionals are often unaware of the use of enzymes in the workplace.

2.6.1.4. Enzyme-producing industry

A Danish company, the largest enzyme manufacturer in the 1960s–1980s, reported sensitization prevalences (by RAST) of 3.3 and 10% for detergent proteases during the 1970s (Witmeur et al 1973, Zachariae et al 1981); 3% and 8.5% respectively, experienced respiratory symptoms in conjunction with enzyme exposure. The company published data from its medical surveillance program of employees again in 1997 (Johnsen et al 1997). During the period 1970–1992, 8.8% of the employees developed clinical enzyme allergy during the first 3 years of employment. The frequency was 5.3% for asthma, 3.0% for rhinitis and 0.6% for urticaria. Several enzymes, like amylases, cellulases and lipase, appeared as allergens.

In Finland enzyme production expanded rapidly during the 1980s and 1990s, and the first five cases of enzyme allergy due to *Trichoderma*-derived cellulase and xylanase were reported in 1991 (Tarvainen et al 1991). Thus far, 35 cases of occupational disease due to enzyme exposure in enzyme production have been diagnosed, out of a total workforce of about 500–600 during 1990–2000 (Finnish Register of Occupational Diseases). By far the most common

causative enzyme has been cellulase (27 cases); others have been xylanase, phytase, α-amylase, glucoamylase, protease and pectinase. Occupational asthma was diagnosed in 22, rhinitis (without asthma) in 9, contact urticaria in 10, and conjunctivitis in 2 cases.

2.6.1.5. Other industries

Few reports exist from food industry except the bakeries. In Finland, a cheesemaker was sensitized to powdered microbial rennet and had dyspnea in conjuction with exposure (Niinimäki & Saari 1978). Recently, pectinace and glucanase, used in the preparation of citrus fruits for fruit salads, were reported to cause sensitization and asthma (Sen et al 1998). The first case report of cellulase allergy in the textile industry was published by Kim et al (1999). In Finland, a case of occupational asthma due to cellulase in jeans finishing was diagnosed in 2000 (Finnish Register of Occupational Diseases). Phytase, taken into use recently, was reported to cause sensitization in 8 of 11 exposed workers in the animal feed industry (Doekes et al 1999) and asthma in one worker in an animal feed factory (O'Connor et al 2001).

2.6.2. Dermatitis due to enzymes

A recent review presents dermatological symptoms induced by exposure to enzymes (Kanerva & Vanhanen 2000). In the detergent industry, irritant dermatitis was common in the late 1960s, but allergic findings were rare (McMurrain 1970, Göthe et al 1972, Zachariae et al 1973). Case reports have been published of urticaria and protein contact dermatitis due to exposure to α-amylase in bakeries (Schirmer et al 1987, Morren et al 1993) and due to exposure to α-amylase, cellulase and xylanase in the enzyme manufacturing industry (Tarvainen et al 1991, Kanerva et al 1997, Kanerva et al 1998, Kanerva & Vanhanen 1999). In most of these cases, the sensitization was proved with SPTs. Few of the cases showed positivity for both the SPT and the patch test (Schirmer et al 1987, Morren et al 1993, Tarvainen et al 1991).

2.6.3. Allergy to enzymes among consumers

Enzyme-containing pancreatic extracts, used as a medication for patients with cystic fibrosis, were reported to cause sensitization and asthma in the parents of children with cystic fibrosis (Dolan & Meyers 1974, Sakula et al 1977), and also in a dog owner who gave the drug to the pet (Warren & Dolovich 1986).

Table 2. Allergies to enzymes in detergent industry

Study protocol	Testing of sensitization (to bacterial proteases)	Sensitization	Symptoms	Reference
28 selected workers of a detergent plant tested	SPT	21 SPT positive	25 had respiratory symptoms; 20 of them SPT-positive	Flindt 1969, 1996
5 selected workers	Scratch test	All were positive	All had work-related asthma	Wüthrich & Ott 1969
102 workers tested	SPT	34 (37%) SPT positive	14 had allergic symptoms at work (10 with asthma, 3 with rhinitis, 1 with skin eruption)	Shapiro et al 1970
121 workers (all but 8) of a detergent plant	SPT 0.1, 1 and 10 mg/ml	48 (40%) SPT positive Positive reactions to enzymes in 64% of the atopic (by SPT) and 33% of the nonatopic subjects	Cough in 17% of the enzyme-positive and in 12% of the enzyme-negative; dyspnea in 25% and 19%, respectively; 44% of the sensitized and 14% of the unsensitized subjects had an FEV1.0/FVC ratio below 70	Greenberg et al 1970
271 (98%) of a plant population	SPT at 1% and 5%	57 (21%) SPT positive Of enzyme positive 65.5% and of enzyme negative 21.4% were atopic by SPT	42 of the 57 SPT-positive had symptoms of acute chest disease; highly significant association between SPT and respiratory symptoms; positive SPTs most prevalent among mixers	Newhouse et al 1970
Test results of 1727 employees in the Procter and Gamble Co. plant	Intradermal testing at 0.01 mg/ml	1727 employees: 588 had positive intradermal test; in subjects working in areas where concentrated enzyme products were handled, up to 50% were test positive	Among about 3500 employees, there had been 207 respiratory cases (rhinitis, pharyngitis, cough, asthma) and 110 cases of enzyme dermatitis since the onset of production in 1966	McMurrain et al 1970
238 workers tested	SPT	113 (47%) SPT positive	66 had allergic symptoms at work (35 with asthma, 5 with rhinitis, 26 with both rhinitis and asthma; 56 of them were SPT positive to enzyme	Slavin et al 1971
Plant A: 50 out of 125 workers were selected: 20 in highest exposure, 15 with moderate exposure, 15 with low or no exposure	A: Intradermal skin testing at 0.01 mg/ml-0.1 mg/ml	A: 15 (53%) in the moderate exposure group and 9 (45%) in the high exposure group	A: none	Weill et al 1971
Plant B: Random selection, 20 workers in each of 3 groups	B: SPT at 10 mg/ml	B: 3 (16%) in low exposure group, 20 (35%) in moderate exposure group, 11 (52%) in high exposure group	B: 13 (22%) had asthma-like symptoms	

Two Swedish detergent factories; 64 employees investigated	SPT, RAST	8 (17.3%) of 46 exposed workers SPT positive, 7 of them RAST positive	50% of exposed people had cough with exposure to enzymes; 5 of them were sensitized; 47% had burning and itching of skin, but only one was sensitized to the enzyme (urticarial symptoms)	Göthe et al 1972
Total of 1642 workers surveyed in 1968–1975; exposure grouping to high, intermittent high, medium and low groups.	SPT	In high exposure, SPT-positivity in40% of nonatopics and in 75% of atopics; in intermittently exposed, 4.5% versus 20%; the conversion declined steadily (e.g., 41% of the nonatopics were sensitized in the the high exposure group in 1968–1969, 29% in 1969–1971 and 10.5% in 1971–1973, respectively	62 (3.2%) workers had experienced symptoms of "enzyme asthma"; the incidence had diminished strongly since 1972	Juniper et al 1977, Juniper et al 1984
A detergent factory that used only encapsulated Esperase® protease, since 1978, 2 years before the study	RAST	24 workers (15 exposed, study 9 unexposed) tested serologically (RAST); 3 of the exposed were positive	None	Liss et al 1984
731 workers in 5 detergent factories surveyed in the United Kingdom over a period of 4–20 years	Not reported		Among the several thousand employees in the five factories since 1968, 166 confirmed cases of enzyme asthma had been recorded; since 1978, 16 cases had been reported	Cathcart et al 1997
8-year survey of 256 employees in one detergent factory and 216 employees in another	SPT	Over 8 years since 1986, 2.0–2.9% new protease sensitizations yearly; since 1990, the yearly skin test positive rate has averaged 1.3% at both sites	Since 1986, 5 cases of enzyme rhinitis in one plant and one case of rhinitis and one of asthma in another plant	Gaines 1994
Review of allergies in the Procter & Gamble detergent industry	SPT	In 1984–1994, sensitization for protease up to 10% and up to 5% for α-amylase	No new cases of occupational asthma among thousands of workers in North and Latin America since 1994	Schweigert et al 2000
Cross-sectional study in a modern detergent factory, 342 workers tested	SPT with 1mg/ml detergent enzyme solutions (protease, cellulase, α-amylase)	26% sensitized; reactions towards all enzymes (protease, cellulase, α-amylase)	Work-related upper-respiratory symptoms, accompanied by sensitization in 19%, and lower respiratory symptoms in 16%	Cullinan et al 2000

Abbreviations: SPT: skin prick test, RAST: radio allergo sorbent test, $FEV_{1.0}$: forced expiratory volume in 1 second, FVC: forced ventilation capacity.

Table 3. Allergies to enzymes in bakeries

Study protocol	Testing of sensitization	Sensitization	Symptoms	Reference
118 German bakers: 91 screened at random for symptoms and 27 with work-related respiratory or conjunctival complaints	RAST	34 % (12/35) of the symptomatic group and none in the non-symptomatic group were sensitized to amylase	The study group consisted partly of symptomatic bakers	Baur et al 1986
140 German bakers suffering from work-related asthma, rhinitis or conjunctivitis were tested	RAST	24% to amylase; 5% to glucoamylase; 8% to hemicellulase; 1% to papain; 1% to protease; 21% to soy bean flour	The study group consisted of symptomatic bakers	Baur et al 1988
Cross-sectional study among 20 Swedish workers in a factory producing dough improvers; in addition, 4 index cases with amylase sensitization and asthma or rhinitis described	SPT	30% (6/20) to amylase	Rhinitis in 3 amylase-sensitized workers, verified by nasal challenge	Brisman & Belin 1991
Initial cross-sectional survey of a longitudinal study in 3 large modern British bakeries, a flour packing factory and three mills; 304 workers, first employed after a specified date, tested	SPT	5% to amylase; 5% to mixed flour; 17% to *Lepidoglyphus destructor*	Work-related chest symptoms in 14%, eye/nose symptoms in 29%, skin symptoms 9%; there was an association between sensitization (amylase, flour) and exposure, no correlation between sensitization and symptoms	Cullinan et al 1994
Cross-sectional study among 226 bakers and pastry makers from 105 small businesses in Italy	SPT	7.5% to amylase; 11.9% to wheat flour; 17.7% to storage mites; sensitization was significantly associated with atopy, cigarette smoking and seniority	Asthma in 4.9% and rhinitis in 13.7%; significant association with sensitization to occupational allergens	De Zotti et al 1994
Cross-sectional study among 178 bakery workers in 14 Dutch bakeries	SPT, EIA	Amylase SPT/EIA 9% / 8%; wheat flour SPT/EIA 8% / 5%	Symptoms in 25%: chest tightness in 5%, rhinitis in 15%, skin symptoms in 11%, conjunctivitis in 6%; exposure-sensitization relationship noted; atopy was associated with sensitization but smoking was not	Houba et al 1996
A) 89 workers from bakeries screened and	SPT, EAST	Group A: amylase SPT/EAST 19% / 19%; wheat flour SPT/EAST 16% / 53%; rye flour 11% / 34%	46% of group A reported at least one work-related symptom; rhinitis and dyspnea by more than 33%	Baur et al 1998a

B) 104 workers filing a claim for a compensation of baker's asthma		Group B: amylase SPT/EAST 24% / 12%; wheat 47% / 62%; rye 37% /50%	In group B, 90% had rhinitis, 50% had asthmatic symptoms, and 60% had conjunctivitis	
Sera of 171 bakers complaining of work-related respiratory symptoms screened retrospectively	EAST	23% to amylase; 8% to glucoamylase; 13% to cellulase; 11% to xylanase	A new allergen, *Aspergillus niger*-derived β-xylosidase (Asp n 14) identified	Sander et al 1998
293 workers in 19 bakeries and 77 cakebakers in 3 bakeries in the United Kingdom	SPT	16% to amylase and 6%to wheat flour in bread bakeries versus 1% and 3% in cake bakeries	Work-related asthma in 0.5% of bread bakers versus 0% of cake bakers; work-related rhinitis 2.6% versus 0%	Smith & Smith 1998
Cross-sectional study in 18 small bakeries in Scotland; 224 workers investigated	RAST testing to 205 workers	15% sensitized to amylase, versus 24% to wheat flour and 16% to rye	Work-related asthma-like symptoms in 20.9%; at least eye, nasal or lower airway symptoms in 43.7%; significant association between work-related symptoms and sensitization to flour or amylase	Jeffrey et al 1999
33 large modern bakeries, 3 flour mills and one packing station in the United Kingdom; 264 employees for epidemiological analyses, divided into 3 amylase exposure categories: arithmetic mean < 5 ng/m^3, 5–15 ng/m^3 and >15 ng/m^3	SPT	5% sensitized to amylase	None had work-related chest symptoms, one had eye and nose symptoms and one skin symptoms; significant exposure-response relation found between exposure and sensitization; atopics had an increased risk of sensitization	Nieuwenhuijsen et al 1999
A cohort of Italian trainee bakers: 125 subjects tested at 6, 18 and 30 months after the baseline examination	SPT	At the baseline, 4 sensitized to flour or amylase; at 30 months, 10 sensitized to flours and 3 of them also to amylase	The cumulative incidence of work related respiratory symptoms was 4.8% at 18 months and 9.0% at 30 months; the symptoms were significantly associated with personal history of allergic disease and sensitization to flour or amylase, but not with atopy by SPT	De Zotti et al 2000
A nested case-control analysis for a cohort of new employees in the United Kingdom baking industry (see Cullinan et al 1994 for initial study); average period of follow-up 3.5 years; altogether 300 employees	SPT	Incidence of sensitization to amylase: 2.5 cases/100 py; to flour 2.2/100 py; positive exposure-sensitization relationship	Incidence of 11.8/100 py for work-related eys/nose symptoms; 4.1/100 py for chest symptoms; positive exposure-symptoms relationship; incidence of work-related chest symptoms in the presence of positive SPT to flour or amylase: 1/100 py	Cullinan et al 2001

Abbreviations: SPT: skin prick test, RAST: radio allergo sorbent test, EIA: enzyme linked immunoassay, EAST: enzyme-allergosorbent test.

Table 4. Allergies to enzymes in other industries

Industry	Study protocol	Testing of sensitization	Sensitization	Symptoms	Reference
Enzyme production	Cross-sectional study in two factories of Novo Nordisk A/S in Denmark, 355 people in study group	RAST to 211 people	3.3%	27 people displayed signs of "enzyme dermatitis", 12 had cough and 6 chest tightness at enzyme exposure	Witmeur et al 1973
	A survey in enzyme production at Novo Nordisk A/S during 1970–80: 667 workers	RAST	31 (4.6%) and 70 (10%) out of 667 workers sensitized to Esperase® and Alcalase®, according to RAST tests	22 workers reported respiratory symptoms (16 asthma-like symptoms)	Zachariae et al 1981
	Cross-sectional study in an enzyme-producing plant in the United States; 36 people (65% of work-force) tested	SPT	50% reacted to alkaline protease (supposed to be irritant effect); 22% to glucoamylase, 22% to amylase	Itchy eyes in 36%, chest tightness in 31 %, cough in 28%, runny nose in 25%, flu-like sensation in 28%, fever in 17%	Biagini et al 1996
	Retrospective follow-up study of 1064 workers at Novo Nordisk A/S in Denmark during 1970–1992	RAST	36% had a RAST value above detection limit of 0.5 SU and 8% > 2SU; sensitisation occurred to all tested enzymes: amylases, proteases, cellulases, lipases; smoking was a risk factor for sensitization; atopy was not, but selection may have had a role	8.8% developed clinical enzyme allergy during the first 3 years of employment: asthma in 5.3%, rhinitis in 3.0%, urticaria in 0.6%; the prevalence of allergy declined during 1970–1992: 13% in 1970–1979, 9.5% in 1980–1986, and 6.1% in 1987–1992	Johnsen et al 1997
Pharmaceutical industry					
Chymotrypsin, trypsin	A case report: two laboratory workers	SPT	Both sensitized	One had conjunctivitis and allergic rhinitis, another was symptomless	Howe et al 1961
Papain	A case report: four food technologists	Scratch test	Tests made to two of the four: positive	One had rhinitis, three had dyspnea	Milne & Brand 1975
Bromelin	A case reports: a laboratory worker and a messenger boy from a pharmaceutical plant	SPT	Both sensitized	Both had asthma	Galleguillos & Rodriguez 1978
Papain	33 workers screened: kitchen workers handling papain as a meat ternderizer; workers packing papain	SPT, RAST	16 SPT positive, 15 of whom also RAST positive	Work-related symptoms in 17: dyspnea in 15, rhinitis in 15, conjunctivitis in 5, flare reactions of skin in 3	Baur et al 1982
Pectinase	A case report: two workers from a company handling pectinase	Scratch test, RAST	Both sensitized	Both developed asthma	Hartmann et al 1983
Pepsin	A case report: a worker from a pharmaceutical company processing hog and beef stomach extracts	SPT, RAST	SPT and RAST positive	Deterioration of previous asthma and allergic rhinitis at work	Cartier et al 1984

Pancreatic extracts	14 selected workers from a pharmaceutical company handling porcine pancreatic extracts	SPT	All were sensitized	All had dyspnea, two also symptoms indicating alveolitis	Wiessmann & Baur 1985
Cellulase from *Aspergillus niger*	A case report: two workers from a pharmaceutical firm manufacturing digestive aids; powdered enzyme used	SPT, REIA	Both sensitized by SPT and REIA	Asthma in both patients	Losada et al 1986
α-Amylase from *Aspergillus oryzae*	83 workers from pharmaceutical industry exposed to powdered amylase	SPT, REIA	26 (31%) sensitized by SPT; exposure-response relationship by exposure assessment	20 out of 26 sensitized had symptoms of rhinitis and/or asthma	Losada et al 1992
Egg lysozyme	A case report: a worker in a company manufacturing egg lysozyme powder for use in the pharmaceutical industry	SPT, ELISA	One worker sensitized	Asthma	Bernstein et al 1993
Serratial peptidase and lysozyme	A case report from pharmaceutical industry	SPT, ELISA	One worker sensitized	Asthma	Park & Nahm 1997
Lactase	Cross-sectional survey of 207 pharmaceutical workers handling powder-form lactase	SPT	31% sensitized to lactase; atopics more likely to be sensitized	Sensitization correlated with upper but not lower airway symptoms	Muir et al 1997
Lactase	Cross-sectional survey of 94 pharmaceutical workers handling powder-form lactase	SPT	29% sensitized to lactase; atopics 4 times more likely to be sensitized	The sensitized people were 9 times more likely to have work-related respiratory symptoms	Bernstein et al 1999b
Fruit salad processing: pectinase and glucanase	Case report: three workers handling liquid pectinase and glucanase	RAST	All were RAST positive to pectinase and glucanase	All three developed asthmatic symptoms at work within 6 months and improved following withdrawal	Sen et al 1998
Animal feed industry:					
Phytase	Cross-sectional study in a factory producing enzyme premixes for animal feed industry;11 exposed workers studied	EIA	Four reacted definitely and four had a borderline reaction	Six had work-related respiratory symptoms; most of these were sensitized to phytase	Doekes et al 1999
β-glucanase, phytase	Case report: a director of an animal feed manufacturing plant	SPT, RAST	SPT and RAST positive to both enzymes	Asthma	O'Connor et al 2001
Textile industry: cellulase	Case report: a textile company worker using cellulase to remove fuzz from clothes	SPT, ELISA	SPT and serum specific IgE positive	Asthma	Kim et al 1999

Abbreviations: EIA : IgE enzyme immunoassay, ELISA: enzyme linked immunosorbent assay, RAST: radioallergosorbent test, REIA: reverse enzyme immunoassay, SPT: skin prick test, SU: sorbent units.

Allergies in the detergent industry coincided with the emergence of allergies in consumers of detergents (Belin et al 1970, Bernstein 1972, Zetterström & Wide 1974). With the decrease of enzyme addition in the formulations and the use of encapsulated preparations, the allergies ceased (Pepys et al 1973, White et al 1985, Sarlo et al 1996). Contact urticaria has been reported as a result of exposure to papain in cleansing solutions for contact lenses (Bernstein et al 1984, Santucci et al 1985). Recently a detergent company published an experiment in which volunteers used a shower gel that contained protease enzyme. Because of the detection of protease in the shower aerosol and the appearance of sensitization to protease in the test persons, the company decided not to add enzymes to its shower gel products (Kelling et al 1998).

A case report report described a severe systemic allergic reaction after ingesting meat tenderizer that contained the proteolytic enzyme papain (Mansfield & Bowers 1983). Allergy to α-amylase in bread has been suggested in two case reports showing that eating bread baked with the aid of amylase caused allergic symptoms in two previously occupationally (by inhalation) sensitized individuals (Kanny & Moneret-Vautrin 1995, Baur & Czuppon 1995). It was also demonstrated that bread contained residual amounts of antibody-binding α-amylase that was not destroyed by the baking process (Baur et al 1996, Sander et al 2000).

2.6.4. Determinants of sensitization

Exposure-response relationships in the detergent industry were first assessed by Weill et al (1971). The risk of sensitization increased along with the exposure in three groups of workers, the groups being formed according to estimated (work task) and monitored exposure to enzymes. In bakeries, Houba et al (1996) showed a strong positive association between measured α-amylase exposure levels and amylase sensitization. α-Amylase exposure levels above 0.25 ng/m^3 as an average exposure during an 8-hour work shift increased the risk of sensitization of bakery workers. In another bakery study, a significant exposure-response relationship was noted between exposure and sensitization in three exposure groups (< 5 ng/m^3, 5–15 ng/m^3 and >15 ng/m^3) (Nieuwenhuijsen et al 1999).

Atopy has been shown to be a strong determinant of sensitization to enzymes in most studies, atopics (determined usually by SPT) being up to 4–5 times more prone to sensitization (Brisman 1994, Bernstein et al 1999a). Smoking, on the other hand, has been shown to be a risk factor only occasionally (De Zotti et al 1994, Johnsen et al 1997).

2.7. Characterization of enzyme allergens

The most thoroughly analyzed industrial enzyme is α-amylase derived from *A.oryzae.* Several proteins that bind to immunoglobulin E (IgE) have been detected in crude enzyme preparations, the dominating band having a molecular weight (MW) from 51 to 54 kDa (Quirce et al 1992, Baur et al 1994, Sandiford et al 1994, Houba et al 1997). The allergens were further studied, purified and identified (Baur et al 1994). A protein with a MW of 53 kDa was shown to represent the dominating allergen. The enzyme is a 478 amino-acid glycoprotein. The allergen was named *Asp o* 2. A xylanase from *A. niger*, used in baking additives, was shown to be allergenic, the allergen being β-xylosidase of 105 kD (Sander et al 1998).

Kim et al (1999) demonstrated that a cellulase preparation derived from *T.viride* and *Fusarium moniliform* had at least eight IgE binding components, the strongest band being at 56–63 kDa.

The structure of an increasing number of environmental allergens has been determined (Aalberse 2000, Liebers et al 1996). Many of the allergens are functionally enzymes, for example, the allergens of flour, house dust mite and molds (Liebers et al 1996, Tiikkainen et al 1996, Houba et al 1998a, Sander et al 2001, Robinson et al 1997, Lake et al 1991, Robinson et al 1990). The proteolytic function of many of these allergens has been proposed to be an important factor in the epithelial permeability and origin of allergy (Robinson et al 1997, Kauffman et al 2000). Sandiford et al (1994) showed cereal amylases to be important allergens in patients with allergy to flour, but only minimal cross-reactivity was found between cereal amylases and fungal a-amylase.

2.8. Diagnosing enzyme-induced asthma with a challenge test

Enzymes cause the following clinical symptoms and diseases typical of type I hypersensitivity: asthma, rhinitis, conjunctivitis, and urticarial skin symptoms. Guidelines have been introduced for the diagnostics of occupational asthma (Subcommittee on Occupational Asthma of the EAACI 1992). The recommended five steps were as follows: (1) history suggestive of occupational asthma, (2) confirmation of asthma, (3) confirmation of work-related bronchoconstriction with serial measurements of peak expiratory flow rate (PEFR) and confirmation of non-specific bronchial reactivity, (4) confirmation of sensitization to occupational agents, and

(5) confirmation of the causal role of the occupational agent with specific bronchial challenges.

The bronchial challenge test is regarded as the gold standard in the diagnosis of occupational asthma (Pepys & Hutchcroft 1975, Nordman 1994a, Chan-Yeung & Malo 1995, Cartier 1998, Cartier & Malo 1999). It is superior to PEFR in specificity and preferred especially when there is uncertainty about the causative agent or the agent is a "new" sensitizer or the patient history indicates severe symptoms, and uncontrolled PEFR monitoring is not regarded as being as safe as a controlled challenge test.

Challenge tests with enzymes have been performed with a variety of protocols (Table 5). Basically, there are two different methods. One is to generate an aerosol or dust and inhale it through a special device. Another is a protocol in which the substance to be inhaled is generated into the free space (in a challenge chamber), where the subject inhales the dust.

2.9. Monitoring of enzymes in the workplace air

Since the late 1960s, methods to determine airborne enzymes have been in use, first in the production of proteases and in the use of proteases in the detergent industry and, since the late 1980s, in bakeries. Proteases have been measured with catalytic methods in detergent factories (Newhouse et al 1970, Weill et al 1971, Juniper et al 1977, Bruce et al 1978, Liss et al 1984) and, gradually, with immunologic methods (Agarwall et al 1986, Gaines 1994, Cathcart et al 1997, Kelling et al 1998). In the baking industry α-amylase has been measured with catalytic methods (Brisman & Belin 1991, Jauhiainen et al 1993) and later with immunologic methods (Houba et al 1996, Sander et al 1997, Burstyn et al 1998, Nieuwenhuijsen et al 1999, Elms et al 2001).

2.9.1. Catalytic methods

The catalytic methods for measuring enzymes are based on the specific enzymatic function of the enzyme in question; accordingly, only active enzyme is measured. Air samples are filtrated through glassfiber filters using high-volume samplers, followed by the analysis of filter eluates for their ability to hydrolyze the substrate (Dunn & Brotherton 1971, Rothgeb et al 1988, Jauhiainen et al 1993). Assays have also been developed for real-time monitoring of some protease enzymes in workplace air (Tang et al 1996).

Table 5. Challenge tests with enzymes

Enzyme	Method	Subjects	Results	Reference
	1. Aerosol			
Bovine and porcine pancreatic trypsin	Aerosol generated with a DeVilbiss nebulizer; vital capacity and maximum midexpiratory flow rate recorded	One worker at the extraction of crude trypsin powder preparations	Immediate asthmatic reaction	Zweiman et al 1966
Protease Alkalase®	Aerosol through a Wright's nebulizer; concentration of 0.1–1.0 mg/ml; $FEV_{1.0}$ followed	3 workers from a detergent plant	Both immediate and late (4–5 hours) asthmatic reactions	Pepys et al 1969
Protease Alkalase®	Aerosol through a DeVilbiss nebulizer; exposure period 1 minute; concentration chosen from 0.01 to 10 mg/ml; follow-up for 24 hours	29 workers from a detergent plant	Bronchial reactions in 7: 6 immediate, one after lapse of 4–6 hours, 4 dual reactions	Mitchell et al 1971
Papain	Papain solution inhaled through nebulizer, starting from concentration of 0.001 mg/ml; airway resistance monitored by plethysmograph for 5 hours	9 workers who had handled papain in spice mills or kitchen work	Bronchial reactions in 8: 5 immediate reactions, 3 dual reactions	Baur et al 1982
α-Amylase from *Aspergillus oryzae*	Aerosol through a DeVilbiss 646 nebulizer; the starting solution was decided according the concentration of positive SPT solution; $FEV_{1.0}$ follow-up for 24 hours	One baker	Immediate asthma reaction 5 minutes after inhaling a 10^{-4} solution	Blanco Carmona et al 1991
α-Amylase from *Aspergillus oryzae* and cellulase from *Aspergillus niger*	Aerosol generated with DeVilbiss nebulizer; tidal breathing for 2 minutes; progressive enzyme concentrations, determined by SPT; $FEV_{1.0}$ follow-up for 2 hours	5 bakers	Immediate or dual responses to amylase (at concentrations wt/vol. 1:1000 –1:100 000) and cellulase (wt/vol. 1:400 –1:1000 000) in 5 patients	Quirce et al 1992
α-Amylase, glucoamylase, protease, cellulase, hemicellulase, pectinase	Aerosol generated with a jet nebulizer; 10 breaths of 10-fold dilutions of 100mg/ml enzyme solutions in intervals of 10 minutes; PEFR recorded for 5 hours	42 workers from an enzyme manufacturing plant	Immediate reactions in 13 subjects	Merget et al 1993

Enzyme	Method	Subjects	Results	Reference
	1. Aerosol			
α-Amylase	Aerosol generated with DeVilbiss 646-nebulizer; amylase extract from 1:1000 000 to 1:100 wt/vol. used; FEV1.0 follow-up for 8 hours	2 bakers	Both had immediate asthmatic responses after challenge: one with 1:10 000 and one with 1:100 000 wt/vol. solutions	Valdivieso et al 1994
α-Amylase	Aerosol from DeVilbiss nebulizer, concentration determined by the positive SPT solutions; tidal breathing for 2 minutes; $FEV_{1.0}$ follow-up for 10 hours	3 bakers	All had immediate asthmatic reactions	Alvarez et al 1996
Xylanase from *Aspergillus niger*	Aerosolized xylanase, a nebulizer used	One baker	Asthmatic reaction after application about 0.5 µg of xylanase (10 breaths of a xylanase concentration of 1µg/mL)	Baur et al 1998b
Cellulase from *Trichoderma viride* and F*usarium moniliform*	Enzyme extracts 0.1, 1.0 and 2.5 mg/ml; nebulizer + DeVilbiss dosimeter, $FEV_{1.0}$ follow-up for 7 hours	One textile worker	Immediate asthmatic reaction after dose of 2.5 mg/ml	Kim et al 1999
	2. Enzyme in capsules			
Cellulase from *aspergillus niger*	Capsules with 99.90 mg of lactose + 0.1 mg of cellulase, and 99.50 mg of lactose+ 0.5 mg of cellulase; capsules inhaled through a hand-held patient-activated turboinhaler; $FEV_{1.0}$ follow- up for 24 hours	2 workers from a pharmaceutical firm manufacturing digestive aids	Immediate asthmatic reaction in both patients with 0.5 mg of cellulase; 0.1 mg negative	Losada et al 1986
α-Amylase from *Aspergillus oryzae*	Powdered amylase mixed with lactose in capsules, inhaled through a turboinhaler; $FEV_{1.0}$ follow-up for 24 hours (see Losada et al 1986)	14 workers	6 immediate asthmatic responses	Losada et al 1992
	3. Enzyme dust			
Protease Alkalase®	50 g of the test substance (enzyme-free detergent and 0.3% and 1% Alkalase detergents) placed in a bowl; the patient poured the substance from the bowl to another bowl for 5 minutes; PEF measured; test repeated after 15 minutes	12 workers from a detergent factory	Rhinitis in 8, conjunctivitis in 1, bronchial reaction in 3; in 6 patients the reactions were achieved with 0.3% Alkalase®	Zetterström 1977

Pepsin	The patient poured powdered papain from one tray to another for 15 minutes; $FEV_{1.0}$ recorded for 8 hours	One worker from a pharmaceutical company	Immediate asthmatic reaction	Cartier et al 1984
Lysozyme	The patient sifted 50 mg of powdered enzyme back and forth between porous trays; lactose powder as placebo test; $FEV_{1.0}$ and FVC recorded; for possible late reaction, a mini-Wright flowmeter for home measurements	One worker from pharmaceutical industry	Immediate asthmatic reaction	Bernstein et al 1993
α-Amylase from *Bacillus licheniformis*	Granulated enzyme crushed with pestle and mortal and exposed with dust tipping method; no details given about dose or exposure time; lactose powder as inert control	4 workers from a detergent factory	Rhinitis and asthmatic responses in all: one late (6 hours) and 3 dual responses	Hole et al 2000
Phytase, β-glucanase, α-amylase	The patient poured powdered enzyme from cup to cup near his face for 3 minutes; spirometry recorded	One worker sensitized to phytase and β-glucanase	Rhinitis and asthmatic symptoms from phytase and β-glucanase but not α-amylase	O'Connor et al 2001
	4. Nasal challenge			
α-Amylase from *Aspergillus oryzae*	0.2 ml of amylase solution at a concentration of 1.0 mg/ml was sprayed into a nostril; continued with a 10-fold increase until a positive reaction was achieved or until a concentration of 100 mg/ml was reached	6 workers from a plant producing bread improvers	Positive in 3 workers	Brisman & Belin 1991
α-Amylase from *Aspergillus oryzae*	1:100 wt/vol. solution of amylase; 0.05 ml inserted up one nostril and 0.05 ml of dissolvent up the other nostril; symptoms observed after 30 minutes and up to 8 hours	11 workers	6 immediate positive	Losada et al 1992
α-Amylase	Dilutions of amylase extract (from 1:100.000 to 1:100 wt/vol.) were sprayed in each nostril using a DeVilbiss spray	2 bakers	Both had positive responses, as assessed by number of sneezes, amount of secretion and decrease in nasal peak flow: one to 1:100 wt/vol. and one to 1:100.000 wt/vol.	Valdivieso et al 1994

Abbreviations: $FEV_{1.0}$: forced expiratory flow in one second, PEFR: peak expiratory flow rate, SPT: skin prick test, wt/vol: weight per volume.

2.9.2. Immunologic methods

Immunologic methods are based on enzyme-specific antibodies. Agarwall et al (1986) were the first to report an immunologic method for quantitating a protease (Esperase®) in the detergent industry. The method used a two-site immunoradiometric assay and had a great sensitivity (1 ng/m^3). Another method utilizes enzyme-linked immunosorbent assay (ELISA) in the immunodetection of detergent protease and cellulase (Miller et al 1990, Miller et al 1994, Kelling et al 1998). The detection limit was as low as 0.2–0.5 ng/m^3.

Immunochemical methods for α-amylase in bakeries have been reported since 1996. Houba et al (1996) developed a method in which the enzyme is detected using sandwich enzyme immunoassay (EIA). Polyclonal anti-amylase antibodies were used. The detection limit for amylase allergen measurement in personal sampling was as low as 0.25 ng/m^3. This method was used in measurements in bakeries in The Netherlands (Houba et al 1996, 1997), the United Kingdom (Nieuwenhuijsen et al 1999) and Canada (Burstyn et al 1998). In Germany, a two-site monoclonal antibody ELISA was developed to quantify the allergen Asp o 2 (α-amylase from *A.oryzae*). The assay used two monoclonal antibodies and had a sensitivity of 0.6 ng/ml (Sander et al 1997). Another method based on monoclonal antibodies was reported by Elms et al (2001), with a sensitivity of 0.2 ng/ml. The authors could also monitor short (15 minute) exposures, detecting short peak exposures that are easily overlooked with longer sampling times.

A comparison of four immunologic methods for assessing α-amylase was reported by Lillienberg et al (2000). Three assays used polyclonal antibodies (Houba et al 1996, Sander et al 2000, Lillienberg et al 2000) and one employed monoclonal antibodies (Sander et al 1997). The three methods using polyclonal antibodies showed good agreement, with a factor of less than 2 between the methods for individual samples. The method with monoclonal antibodies showed 3–6 times higher values for individual samples.

Substantial benefits have been proposed for the immunologic methods, as compared with the catalytic methods. They detect the specific enzyme protein in all instances, whether the enzyme is active or inactive, which is beneficial, as inactivated enzyme proteins (or parts of proteins) may still act as allergens. Second, the immunologic assay is more specific. In bakeries, for example, the catalytic method also measures the activity of inherent cereal amylase. Accordingly, the assay of Houba et al (1996) appeared to be highly, although not totally specific for fungal amylase. The assays using monoclonal antibodies (Sander et al 1997, Elms et al 2001) were even more specific. Very high concentrations of wheat flour, rye flour, yeast

proteins and storage mite allergens did not increase the background value.

The reported measurements of enzyme levels in industries are summarized in Tables 6–7. As is apparent from the data, it is often difficult to compare the concentrations between workplaces due the different enzymes used, the different monitoring and detecting methods used, and the different units in use to express the enzyme activity (in catalytic methods). Detailed data on monitoring results in the enzyme-producing industry and the detergent industry have not been published, but it has been reported that a major shift has occurred from levels of tens or hundreds of micrograms (Weill et al 1971) to levels under the adopted exposure guideline of 15 ng protein/m^3 in the detergent industry (Gaines 1994, Cathcart et al 1997, Cullinan et al 2000, Schweigert et al 2000). In bakeries, recent measurements with immunologic methods have revealed peaks of about 40–300 ng/m^3 in dough making, whereas in other tasks, levels are generally under 10 ng/m^3 (Houba et al 1996, Sander et al 1997, Ståhl et al 1998, Burstyn et al 1998, Nieuwenhuijsen et al 1999, Elms et al 2001).

2.10. Exposure guidelines for enzymes

There is only one threshold limit value (TLV) for industrial enzymes worldwide: 60 ng of pure chrystalline protein/m^3 for subtilisin (a *B.subtilis* protease), established by the American Conference of Governmental Industrial Hygienists (ACGIH 1980). The TLV level was based on experiences in the detergent industry in the late 1960s and early 1970s and on some enzyme concentration data. Later, large detergent companies have adopted occupational exposure guidelines with a limit of 15 ng protein/m^3 (Schweigert et al 2000, Peters et al 2001).

The Soap and Detergent Industry Association (SDIA) (Gilson et al 1976, Schweigert et al 2000) and The Association of Manufacturers of Fermentation Enzyme Products (AMFEP 1994) have published guidelines on the safe handling of enzymes for use by their member companies, as well as for customers.

Table 6. Enzyme air concentrations in in detergent industry

Enzyme	Method	Area/job monitored	Total dust mg/m^3	Enzyme concentration, stationary sampling	Reference
Alcalase®	Catalytic	Enzyme handling and slurry spraying		103 x10^{-6} Anson units/m^3 (average in April 1969)	Newhouse et al 1970
				6 x10^{-6} Anson units/m^3 (average in Oct 1969)	
		Bag filling and packing		11 x10^{-6} Anson units/m^3 (average in April 1969)	
				0.5 x10^{-6} Anson units/m^3 (average in Oct 1969)	
Subtilisin	Catalytic	Average exposures by exposure groups in two plants:			Weill et al 1971
		"Low exposure"		<1 μg/m^3	
		"Moderate exposure"		1-5 (peaks up to 20) μg/m^3	
		"High exposure"		3–30 (peaks up to 1000) μg/m^3	
"Protease"	Catalytic	Packing room	0.66 –22.38	0.66-2.16 μg/m^3 (proteolytic enzyme activity (11.5 Anson Unit))	McMurrain 1970
"Protease"	Catalytic	Packing department	1.2 (1969) 0.2–0.3 (1975)	1.25 glycine units/m^3 (1969) 0.03–0.05 glycine units/m^3 (1975)	Juniper et al 1977
Esperase®	Catalytic	Blending area		Up to 1.57 μg/m^3 (8-hr time-weighted average 0.64 μg/m^3)	Liss et al 1984
		Filling area		Up to 0.76 μg/m^3 (average 0.49 μg/m^3)	

Esperase®	Immunochemical method; two-site immuno-radiometric assay	Processing Packing	*) <4.0–21.0 ng/m^3; average 4.25 ng/m^3 <4.0–180.0 ng/m^3; average <36.3 ng/m^3	Agarwall et al 1986
Alcalase®, Savinase®	Inhibition enzyme immunoassay developed	Air samples "at a detergent manufacturing site"	Alcalase® 0–2.92 (mean 0.76) ng/m^3, Savinase® 0–1.49 (mean 0.2) ng/m^3	Miller et al 1990
"Protease"	Immunochemical methods in use since 1991	No details given	Limited data given; the occupational exposure guideline of 15 ng protein/m^3 was met 98-99% of the time in the two plants reported (1986 to 1993)	Gaines 1994
"Protease"	Not given, probably immunologic	"Average workplace enzyme levels" are given	About 1.0 ng/m^3 (since 1976)	Cathcart et al 1997
Bacillus protease	Not given, probably immunologic	No details given	Geometric mean 4.25 ng/m^3 in 1997; 5% of measurements were above 15 ng/m^3, highest value being 57 ng/m^3	Cullinan et al 2000

*): personal sampling

Table 7. Enzyme (α-amylase) air concentrations in baking industry

Method	Area/job monitored	Total dust mg/m³	Enzyme concentration, stationary sampling	Enzyme concentration, personal sampling	Reference
Catalytic	A factory producing flour additives	6.7 –10 during packing	No detectable amylase activity in mixing	30 μg/m³ during packing	Brisman & Belin 1991
Catalytic	6 bakeries:	(Personal samples)			Jauhiainen et al 1993
	Weighing of flour additives	4.2 ± 1.6 (mean ± SD)		7.3 ± 6.7 μg/m³ (mean ± SD) (range 1.2–21.0)	
	Making of dough	4.6 ± 1.6 (mean ± SD)	1.4 ±1.8 μg/m³ (mean ± SD) (range 0.06–4.7)	0.6 ± 0.6 μg/m³ (mean ± SD) (range 0.1–2.0)	
	Making of bread	2.3 ± 0.9 (mean ± SD)	0.1 ± 0.2 μg/m³ (mean ± SD) (range 0.07–0.5)	± 0.1 μg/m³ (mean ± SD) (range 0.1–0.3)	
Immunologic: sandwich-EIA, polyclonal antibodies	14 bakeries in The Netherlands				Houba et al 1996
	Doughmaking	3.0 (GM)		18 ng/m³ (GM) range 0.2–221.8 ng/m³	
	Other tasks	0.4 (GM)		0.7–1.3 ng/m³ (GM) range 0.2–33 ng/m³	
Immunologic: sandwich-EIA, polyclonal antibodies	5 large industrialized and 16 small bakeries in The Netherlands			Highest exposure in dough makers in large industrial bakeries (GM up to 18 ng/m³; GSD 4.56) In small traditional bakeries, GM 0.3 ng/m³ (GSD 3.09)	Houba et al 1997
Immunologic (see Houba 1996)	5 small bakeries and 2 large bakeries in Canada	0.1–110		<0.1–307.1 ng/m³; in dough making 44.1 ng/m³ (GM)	Burstyn et al 1998

Immunologic: two immuno-assays based on precipitating IgG antibodies from exposed workers – EIP – two-sited sandwich ELISA	A bread improver production plant and 19 bakeries in Sweden		Area samples analyzed by EIP: In bread improver production continuous exposure to amylase detected: range 31–1000 ng/m^3 In bakeries, short peaks of 31–100 ng/m^3	Personal samples analyzed by ELISA: 0.8–253 ng/m^3 (GM 12.1 ng/m^3); the highest concentrations were found in samples of dough makers, half of these being >20 ng/m^3	Ståhl et al [1)]
Immunologic (see Houba 1996)	3 large modern bakeries and 3 flour mills in the United Kingdom			In the bakeries, the highest activities of enzyme were measured in the dispensing and mixing areas (GM 39.7 ng/m^3) and at some of the bread and roll production areas (up to GM 5.8 ng/m^3), however, there were great differences in exposures between different bakeries: GM from 1.4 to 39.7 ng/m^3 in dispense and mixing, 0.4–5.8 ng/m^3 in bread production, and 0.1–4.2 ng/m^3 in roll production; in the flourmills and packing stations the highest exposures to α-amylase were up to 5.4 ng/m^3 (GM)	Nieuwenhuijsen et al 1999
Monoclonal antibody based ELISA; two monoclonal antibodies to α-amylase	4 various size bakeries in the United Kingdom	Personal sampling; inhalable dust levels 0.8–39.8		Inhalable amylase up to 26.5 ng/m^3 (flour mixing; sampling time 1 hour); with sampling time of 15 minutes, peaks up to 190 ng/m^3 (mixing) and 70.5 ng/m^3 (pastries bakery)	Elms et al 2001

[1)] personal communication (data presented at Nordic workshop to decrease allergies in bakery and mill workers, Gothenburg, Sweden, 15–16 Dec 1998).

Abbreviations: EIA: IgE enzyme immunoassay, EIP: electrophoretic immuno-precipitation, ELISA: enzyme linked immunosorbent assay, GM: geometric mean, GSD: geometric standard deviation, SD: standard deviation.

3. AIMS OF THE STUDY

The aims of this study were

1 To assess exposure to enzymes in the following three major enzyme-using industries in Finland: the baking, detergent and animal feed industries, as well as in the enzyme production and

2 to assess the prevalence of sensitization and work-related respiratory symptoms to enzymes in these industries.

4. MATERIAL AND METHODS

4.1. Workplaces and subjects

To investigate exposure and sensitization to enzymes, investigations were carried out in four bakeries, one flour mill, one rye crisp factory, one biotechnical research laboratory, one biotechnical plant, one detergent factory and four animal feed factories. These workplaces represent major users of enzymes. The selection of industries was based on international reports of growing numbers of cases of enzyme allergy, especially in the baking industry, and on the referral of several symptomatic workers from the Finnish enzyme manufacturing industry to the Department of Occupational Medicine in FIOH. In the detergent industry, there had been a paucity of data since the 1970s. The animal feed industry was one of the newest areas to employ enzymes. Altogether 1132 employees were investigated. All employees in the workplaces were asked to participate, and the participation rate was high, well over 90% in all the plants. The workplaces and employees are summarized in Table 8.

The bakeries were typical small or middle-size Finnish bakeries, where powdered form enzyme-containing additives were manually weighed and added to the dough. Local exhaust ventilation was used during flour pouring in only one bakery, and respiratory protective devices were seldom worn. The enzyme in the baking additive was α-amylase, comprising about 0.5% of its total weight. In the flour mill, mixtures of flour and additives for bakery use were produced; powdered cellulase, xylanase and α-amylase were used. Exposure took place mainly in the laboratory. In the rye crisp factory the enzyme was added automatically to the continuously working dough machines, in the surroundings of which exposure was possible for the operators. Mainly cellulase was used. The cellulase content of the dough was less than one-tenth of that of α-amylase in the bakeries.

The detergent factory had been operating since the 1960s; new facilities were built in the mid-1980s. Detergents for laundry and dishwashing were produced in separate departments. At first sight, the factory looked tidy and not dusty, a modern automated plant. However, exposure to enzymes did take place when enzyme was added to the hopper and during the mixing phases in the production of dishwashing detergents. It was also reported that frequent system failures in the process lines led to peaks of detergent dust. Granulated proteases had been used since the 1960s, and lipase and cellulase had been in use for 2–5 years.

Table 8. Workplaces, job groups and employees investigated

Study	Workplaces and jobs studied	Number of workers investigated	Gender		Age		Duration of employment (%)		Atopy by SPT	Smoking
			Female	Male	Mean	Range	≤ 10 y	>10 y	%	%
Baking industry (study I)	4 bakeries	153								
	Baking	76	46	30	42	19–62	26	74	15	30
	Packing	55	43	12	40	19–60	27	73	14	33
	Office work	22	22	0	40	20–59	40	60	23	29
	Flour mill	62								
	Process work	56	16	39	43	29–64	21	79	14	43
	Office work	7	7	0	36	22–51	25	75	21	43
	Rye crisp factory	150								
	Manufacturing work	74	22	52	41	22–63	22	78	24	30
	Packing	58	35	23	42	21–59	23	77	23	34
	Office work	18	15	3	38	20–58	17	83	28	28
Enzyme production (study II)	Biotechnical research laboratory	94								
	Research and laboratory work	79	36	43	39	26–58	80	20	28	34
	Office work	15	15	0	34	26–56	95	5	13	30
	Biotechnical plant	79								
	Research and laboratory work	27	18	9	43	28–53	81	19	31	20
	Enzyme manufacturing	25	8	17	41	27–49	85	15	25	40
	Baker's yeast production	22	5	17	44	35–58	26	74	18	28
	Office work	5	5	0	37		65	35	20	30
Detergent industry (study III)	Detergent factory	76								
	Process work:	40	14	26	42	20–59	40	60	35	35
	Manufacturing	17								
	Packing	7								
	Maintenance	5								
	Laboratory work	6								
	Storage	4								
	Cleaning	1								
	Office work	36	29	7	47	30–60	61	39	33	36
Animal feed industry (study IV)	4 animal feed factories	218								
	Process work	140	17	121	43	21–60	34	66	24	38
	Office work	78	32	46	42	23–59	46	54	31	32
	Total	832	383	449						

All the animal feed factories were owned by the same industrial company. Consequently, the production methods and enzymes in use in the respective factories were by and large identical. Powdered or granulated enzyme premixes had been used for 7 to 9 years, but recently the enzyme addition had been changed to a liquid form. The manufacturing of animal feed comprised large-scale milling and mixing of the components, followed by the pelleting and packing of the products, in largely closed processes. Enzymes comprised only about one millionth part of the end product. Several enzymes were used, cellulases, hemicellulases, β-glucanases, proteases, phytases, glucoamylases and α-amylases. Exposure to enzymes was the most evident during the filling of the silos with enzyme premixes. In addition, disturbances and leaks in the production lines could release enzyme dust into the factory air.

In enzyme production, two laboratories and one enzyme manufacturing plant were studied. In addition, the plant produced baking yeast and the employees involved were also studied. Altogether, the following enzymes were produced or studied: α-amylases, glucoamylase, proteases, glucose oxidase, cellulases, xylanases and phytase. Especially the research on cellulases and xylanases of *T.reesei* origin increased markedly in the mid-1980s. Part of the enzymes were dry dusty preparations, particularly the cellulases. Exposure to enzymes was possible in all phases of production, from research to fermentation, to the drying and packing of the product. When clusters of enzyme allergies appeared in the early 1990s, industrial hygiene improvements were started in both plants.

4.2. Total dust and enzyme measurements

4.2.1. Sampling

The samples for total dust, α-amylase and protease measurement (studies I, III and IV) were taken by a standardized method in the breathing zone of the workers at a flow rate of 2 l/min and by stationary sampling at a flow rate of 20 l/min, using 37–mm Millipore AA filters for the gravimetric determination of the dust. For collecting the cellulase and xylanase enzymes in studies I and IV, high-volume sampling (GMW Handi-Vol 2000) at a flow rate of 25 m^3/h and glass fiber filters (Whatman GF/C) were used. The sampling times were 1–4 hours in the stationary sampling and 2–4 hours in the personal sampling in the bakeries and the animal feed factories and 1–4 hours and 2–4 hours, respectively, in the detergent factory.

4.2.2. Analysis

α-Amylase was analyzed colorimetrically using a commercial standard kit (Merckotest) (Jauhiainen et al 1993). The method gives the amount of active enzyme. The standard curves were obtained from enzymes identical to those used at worksites where the samples were collected. The detection limit, which depends on sample volume, was 0.1 μg/sample.

Protease activity was determined using the modification of the sensitive endpoint assay for airborne proteases from Genencor International (Geiger 1984). The standard was a Durazym® preparation with an activity of 8.39 DPU/g (Durazym Protease Units, Novo Nordisk A/S), and the protein content of the standard was 0.082 mg protein/mg Durazym® (Lowry method). The detection limit for this assay was 0.25 μDPU/ml (i.e., 2.5 μDPU/filter), which equals 20 ng Durazym® protein/filter. The protease concentrations were expressed as nanograms per cubic meter of air based on the enzyme activity per protein content of the Durazym® standard.

Cellulase and xylanase were determined by a method based on polyclonal antibodies, using the dot-blot technique (Hawkes et al 1982). Cellobiohydrolase I (CBH I), which accounts for 60–80% of the cellulase complex of *T.reesei* (Harkki et al 1991), and xylanase pI 9.0, one of the two major xylanases produced by *T.reesei*, were determined and thus served as indicatory enzymes for the cellulase and xylanase complexes in air. Monoclonal anti-CBH I or anti-xylanase pI 9.0 were used. The intensity of the formed color of the sample dots was compared with those of the standard dots. The detection limits were 20 ng/m^3 for CBH I and 2 ng/m^3 for xylanase pI 9.0.

After the original studies (III–IV) some reanalysis studies were done with the samples of the detergent and animal feed factories. Protease was measured from the samples with an immunologic method, using polyclonal antibodies against the commercial detergent protease Savinase®. The protocol was a modification of that described by Houba et al (1997), and the analysis was made by Mr Arne Ståhl in the Sahlgrenska University Hospital, Gothenburg, Sweden.

4.3. Assessment of work-related symptoms

The participants were asked about their work history, history of atopy, smoking habits and work-related respiratory, conjunctival and skin symptoms indicating hypersensitivity. In studies I–III the questionnaire was a modification of sets of questionnaires that had been used previously in several epidemiological studies concerning work-related allergies in Finland. In study IV the questions were taken

from the extensive Finnish Tuohilampi-questionnaire, developed by researchers from the FIOH, the National Public Health Institute and several universities (Susitaival & Husman 1996). The Tuohilampi questionnaire is based on several internationally established questionnaires. The self-administered questionnaire was returned at the SPT examination. The answers were checked by a physician, and missing points were filled out and unclear answers were clarified.

4.4. Assessment of sensitization

4.4.1. Skin prick test

Sensitization was assessed by the use of SPTs. The SPTs were performed and the results scored routinely (Kanerva et al 1991). The test was done on the volar aspect of the forearm. The result was read as the mean of the longest diameter of the weal and the diameter perpendicular to it. A weal diameter of 3 mm or more and equal to or greater than half of that formed by histamine hydrochloride (10 mg/ml) was defined as positive, indicating sensitization.

Several enzymes were tested in studies I–V. A detailed description of the preparation of the test extract is given in publication I. In short, dry commercial enzyme preparations were extracted in 0.1 M potassium phosphate buffer, pH 7.4, and diluted to the Coca solution (0.5% sodium chloride, 0.3% sodium bicarbonate, 0.4% phenol) to achieve a protein concentration of 100 µg/ml. Part of this solution (2.5 ml) was passed through a Millex-GV filter (0.22 µm membrane, Millipore Ltd) into a sterile vial containing 2.5 ml of glycerol to yield a final protein concentration of 50 µg/ml (study I) or 100 µg/ml (studies II–V). The Coca-glycerol solution served as a negative control.

Rye, wheat, barley and oat flours were tested for the bakeries and animal feed plants (studies I and IV). In the same workplaces, also storage mites were tested: *Acarus siro, Lepidoglyphus destruens* and *Tyrophagus putrescentiae* (Allergologisk Laboratorium A/S, ALK, Copenhagen, Denmark).

Atopy was assessed by SPT with the following panel of common environmental allergens: cat, dog, timothy, birch, alder, mugwort, house dust mite (*Dermatophagoides pteronyssinus)* (ALK). A person with one or more positive SPT reactions to environmental allergens was defined as atopic.

4.4.2. Immunoglobulin E measurements

Specific IgE antibodies to enzymes were determined by the radioallergosorbent test (RAST). RAST tests were performed for the enzyme, flour or storage mite to which the SPT was positive. Proteins of commercial enzyme preparations were conjugated to paper discs activated by cyanogen bromide using the method of Ceska et al (1972). Other reagents for the RAST were obtained from Phadebas RAST kits (Pharmacia Diagnostics, Sweden). The results, in kilounits per liter, were based on the RAST reference serum of Pharmacia Diagnostics. Values over 0.35 kU/l were defined as positive, indicating sensitization.

4.5. Characterization of enzyme allergens

The antigenic characteristics of the bacterial and fungal amylases and fungal cellulase were studied using the sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) system, according to a modified method of Laemmli (1970), and Western blotting (Towbin & Gordon 1984).

4.6. Lung function tests and testing bronchial hyperreactivity (study V)

The spirograms were recorded with a Medikro 101 spirometer (Medikro Oy, Finland). Bronchial hyperreactivity was assessed with a histamine challenge test described by Sovijärvi et al (1993) using an automatic dosimetric inhaler (Spira Medikro). PD_{15} [provocative dose of histamine inducing a 15% drop in forced expiratory volume in 1 s, (FEV1)] was calculated. Bronchial hyperreactivity was confirmed if PD_{15} was $\leq$ 1.60 mg.

4.7. Specific challenge tests (study V)

Challenge tests were performed for 11 employees from the enzyme production industry who were referred to FIOH because of suspected occupational disease due to exposure to enzymes.

Four of them were working in the enzyme production departments of an enzyme factory and three in the laboratory of the factory, and four worked in a plant that spray-dried cellulase on a subcontract basis.

Inhalation challenges were carried out with powdered cellulase (Econase CEP®) in a 6–m^3 ventilated exposure chamber. The protein content of the cellulase preparation was 0.77 mg/mg, out of which 70–80% was enzyme protein, according to the information provided by the manufacturer. The cellulase was mixed with lactose powder in varying concentrations. Four different enzyme-lactose mixtures were used in the challenges. These amounts were derived from our previous experiences with challenge tests using fungal α-amylase, corresponding amounts of enzyme protein being aimed at. The achieved air concentrations were calculated to reflect real workplace exposures. At the lowest level, 30 mg of cellulase was mixed with 100 g of lactose, which equals 0.03% in weight. The predicted air concentration of cellulase was calculated to be 1–5 $\mu g/m^3$. At the next consecutive levels, a tenfold increase in cellulase was used up to 3 g of cellulase in the total amount of 100 g of a lactose-cellulase mixture. The maximum exposure, in two cases, was 10 g of cellulase. The mixture was placed in a bowl, and the enzyme dust was generated with serial impacts, every 60 seconds, of pressurized air from a nozzle over the bowl. The placebo test with lactose was carried out with the same procedure for all patients except one, for whom formaldehyde challenge was performed. The challenges lasted for 30 minutes but were interrupted earlier if necessary because of symptoms. PEFR was recorded with a Wright peak flow meter every 15 minutes during the challenge, and afterwards every 1–4 hours until the end of 24 hours. Diurnal peal expiratory flow curves of unexposed days were used for reference.

4.8. Statistical methods

In assessing the significance of the level of exposure, and of atopy, to sensitization to enzymes and in assessing the significance of enzyme sensitization to work-related symptoms, rate ratios and their 95% confidence intervals were calculated in the Results section of this thesis and in study II (SAS Institute Inc. 1990). For testing the trend in the prevalence of enzyme sensitization with exposure in study II, the Cochran-Armitage trend test was used (StatXact for Windows 1995). In study III, the associations between work category, atopy and symptoms were examined using logistic regression models. Odds ratios and their 95% confidence intervals were calculated.

5. RESULTS

5.1. Enzyme and total dust measurements (studies I, III, IV)

The results of the enzyme and total dust measurements are summarized in Table 9.

In the bakeries, the total dust concentrations were generally less than 5 mg/m^3. The highest levels were measured during dough making, the personal sample values of total dust being around 10 mg/m^3. Local exhaust ventilation was used in one bakery, and, accordingly, lower levels (3–5 mg/m^3) of total dust were found there. The α-amylase concentrations were also highest in dough making, up to 6.6 μg/m^3, whereas in bread making they were generally below 0.2 μg/m^3. Cellulase was not detected. Xylanase concentrations of 2–200 ng/m^3 (mean 65 ng/m^3) were found.

In the flour mill, total dust exposure was also high, up to 6.7 mg/m^3, during mixing operations. The α-amylase concentration was up to 1.1 μg/m^3, and the cellulase concentration was up to 180 ng/m^3 at the site where additives were mixed.

In the rye crisp factory, the total dust concentrations were generally less than in the bakeries and the flour mill (mean value 3.1 mg/m^3 for the personal samples and 0.8 mg/m^3 for the stationary samples). The α-amylase levels were also lower, mean value being 0.1 μg/m^3 for personal samples and 0.03 μg/m^3 for the stationary samples. The cellulase concentrations ranged from 25 to 160 ng/m^3 in different phases of the mixing, dough making and bread forming. At the same sites, lower levels (7–40 ng/m^3) of xylanase were measured.

Systematic measurements of the enzyme concentrations in enzyme production and the laboratories could not be obtained at the time of the study. Some cellulase measurements were done by the companies themselves, air concentrations of 40–60 ng/m^3 being detected during the weighing of samples in a laminar flow cabin and in a weighing room. There are no data from work phases in which higher exposure to enzymes was likely earlier, such as the mixing, drying and packing of enzymes. In comparison, measurements in another plant, where spray drying (as in plant B) and the packing of cellulase were performed on a subcontract basis, revealed cellulase concentrations of 6–7 μg/m^3 during packing. The highest concentrations, up to 120 μg/m^3, were measured in a spray-drying hall due to obvious leaks in the conveyor lines.

In the detergent factory, the total dust exposure was clearly less than in the baking industry, ranging from <0.07 to 1.3 mg/m^3 in the personal samples. The protease concentration ranged from <55 to 1300 ng/m^3 in the personal samples and from <4.0 to 1500 ng/m^3 in the stationary samples. The high (1300 and 1500 ng/m^3) results were obtained in a dusty mixing site in the production of dishwashing detergents. In the production of laundry detergents the level was generally below 50 ng/m^3.

In the animal feed factories, the total dust concentrations varied from < 0.1 to 38 mg/m^3. The protease levels followed the total dust levels, irrespective of the use of added enzyme. Accordingly, the highest protease concentrations, up to 2900 ng/m^3 in the stationary samples and 360 ng/m^3 in the personal samples, were measured in the grain receipt stations. In comparison, in several other locations, the protease concentration was below the detection limits of 40 ng/m^3 in the personal samples and 4 ng/m^3 in the stationary samples. Likewise, the highest α-amylase concentration (200 ng/m^3) was detected in the process areas, where also the total dust level was high, up to 7.8 mg/m^3. However, the correlation with total dust was not uniform, and levels of 50–90 ng/m^3 were found (e.g., at bagging and enzyme doser sites) with total dust concentrations of 0.6–11.7 mg/m^3. At the premix handling site, the area samples of xylanase gave values of 0.7–4.5 ng/m^3.

For the assessment of the comparability between the protease measurements, the samples from the detergent and animal feed factories were analyzed with an immunologic assay, the detergent protease Savinase® being used as the standard. In the animal feed factories, only three samples showed Savinase levels slightly over the detection limit (0.07 ng/m^3). There was no correlation with the protease levels obtained by the catalytic method, which showed elevated protease concentrations in various parts of the factories. In the detergent factory, on the other hand, the immunologic method showed values over the detection limit of 4 ng/m^3 in six samples. The highest concentrations were 56 ng/m^3 and 62 ng/m^3, measured at the mixing site in the production of dishwashing detergents. The catalytic method showed the highest protease concentrations at the same sites. The results of the immunologic and the catalytic methods correlated well (correlation coefficient 0.99, $p = 0.0002$) (Figure 1).

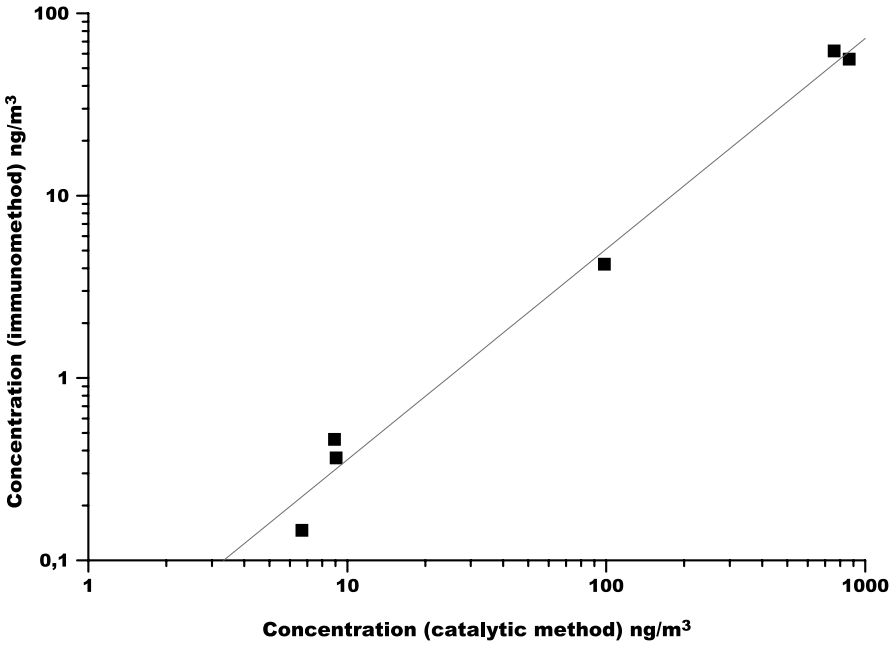

Figure 1. Correlation of the protease measurements using the catalytic method and the immunologic method.

5.2. Sensitization to enzymes (studies I–IV)

The prevalence of sensitization to enzymes in the workplaces is summarized in Table 10.

The prevalence of enzyme sensitization, according to the SPT, was 7.8% for all employees in the bakeries and 11.8% for the bakers' subgroup. The rate was 5.3% (office personnel excluded) in the flour mill, and 3% in the rye crisp factory. The prevalence was 22.5% for the exposed employees' group of 40 persons in the detergent factory, and 7.1% for the process workers' group of 140 persons in the four animal feed factories.

In the biotechnical research laboratory 11.7% and in the biotechnical plant 12.6% of workers were sensitized. In the category of research, laboratory and enzyme manufacturing work, the rates were 12.6% and 15.4%, respectively. The workers were divided into the following three groups according to their estimated exposure to enzymes: "often exposed" comprising workers who had commonly handled both the dry and liquid forms of enzymes or worked often in rooms where dry preparations were handled by others; "occasionally exposed" comprising workers who had handled the

liquid form of enzymes and only occasionally worked in rooms where dry preparations were handled by others; and “rarely or not exposed” made up of workers who did not handle enzymes themselves but who may have worked in laboratories where the liquid form of enzymes was handled. There was a statistically significant ($p=0.003$) exposure-response linear trend, which weakened but remained statistically significant after stratification for atopy ($p=0.01$).

5.3. Sensitization to flours and storage mites

The sensitization to flours was 12% in the bakeries, 6% in the flour mill and 8% in the rye crisp factory. In the animal feed factories the rate was 6.8%. Storage mite sensitization was more common in the animal feed factories (16%) than in the bakeries (9–12%).

5.4. Sensitization to environmental allergens

The prevalence of atopy (Table 8), assessed by SPT, varied from 15–16% (in the bakeries and the flour mill) to 34% (in the detergent factory). Atopy was more common among the office workers than among the process personnel in the baking industry, whereas, in the detergent factory and enzyme laboratories and enzyme producing plant, atopy was as common or more common in the “process” or exposed groups (35% and 29%) than in the “office” or “rarely or unexposed” group (33% and 13.6%, respectively).

5.5. Relation of atopy and smoking to sensitization to enzymes, flours and storage mites

Atopy was significantly associated with sensitization to enzymes in all the workplaces except the detergent factory. Atopics were about 3–5 times more prone to sensitization than nonatopics were (Table 11). Likewise, atopy was significantly associated with flour and storage mite sensitization in the baking and animal feed industries.

Smoking was not associated with sensitization to enzymes in any of the workplaces.

Table 9. Total dust and enzyme concentrations in the baking, detergent and animal feed industries

Industry	Total dust (mg/m³)		α-Amylase (µg/m³)		Cellulase (ng/m³)		Xylanase (ng/m³)	Protease (ng/m³)	
	Personal samples	Stationary samples	Personal samples	Stationary samples	Personal samples	Stationary samples	Stationary samples	Personal samples	Stationary samples
	Mean Range	Mean Range	Mean Range	Mean Range	Mean Range	Mean Range	Mean Range	Mean Range	Mean Range
Bakeries									
Dough making	8.4 (3.0–18.8)	2.5 (0.7–8.4)	2.3 (<0.2–6.6)	1.5 (0.04–4.3)	–	<dl	65 (2–200)		
Bread making	3.2 (1.2–5.5)	1.1 (0.1–2.9)	0.1 (<0.4)	0.3 (<0.02–2.0)	–	<dl	2		
Packing	–	0.1	–	<0.01	–	<dl	2		
Flour mill									
Mixing	5 (3.3–6.7)	1.0 (0.7–1.3)	0.9 (0.7–1.1)	0.07 (<0.02–0.2)	–	110 (65–180)	3 (2–5)		
Laboratory Work	1.8	0.3	<0.6	<0.07	–	–	–		
Rye crisp factory	3.1 (1.0–9.4)	0.8 (0.2–2.1)	0.1 (0.7)	0.03 (<0.02–0.09)	–	85 (25–160)	22 (7–40)		
Detergent factory									
Laundry det.	0.4 (<0.07–1.3)	0.2 (0.05–1.1)						ND (<55–70*)	ND (<4.0–15*)
Dishwashing det.	0.4 (<0.3–1.2)	0.4 (0.1–1.3)						510 (<55–1300)	500 (11–1500)
Animal feed factories									
Control room work	1.0 (<0.1–4.5)	0.5 (0.05–3.5)			ND (<550–<1200)	40 (<36–140)		120 (<38–240))	8.0 (<2.6–19)
Bagging	1.1 (0.3–2.1)	1.6 (0.2–4.2)			520 (<670–700)	280 (<70–820)		210 (<48–750)	ND (<2.5–<6.7)
Grain reception	2.4 (0.6–4.2)	7.4 (0.01–34)			ND <1100	76 (<44–300)		ND 360	490 (<3.2–2900)

Maintenance	5.0 (0.2–38)	–	1700 <900–6700	–	270 (<46–1300)	–
Cleaning	2.4 (0.9–5.4)	–	ND <600–1000	–	ND (<30–50)	–
Preparation of premixes	3.1 (0.3–6.7)	2.4 (0.1–4.5)	480 <900–<1000	340 (<40–660)	ND (<47–<50)	6.7 (4.3–9.0)
Laboratory Work	1.0 (1.0–1.0)	0.2 (0.01–0.4)	–	ND <600	–	8.6 <28
Storage work	0.9 (0.7–11)	–	ND <640–800	–	150 (63–210)	–
Loading of trucks	8.8 (0.4–30)	4.3 (1.1–7.9)	ND <980	140 (142–143)	ND <50	8.3 (<5.8–19)
Work at other process sites	1.4 (<0.1–5.6)	1.1 (0.07–7.8)	1900 <900–9000	220 (<27–3600)	430 (<48–2200)	9.2 (<2.2–41)

– = not done
dl = detection limit
ND = not determined
* Only one result over the detection limit

Table 10. Sensitization to enzymes and work-related respiratory symptoms

Study	Workplaces and jobs studied	Number of workers investi-gated	Positive SPT and RAST to enzymes			Work-related respiratory symptoms, %			Respiratory symptoms in enzyme-SPT positive	Enzymes to which sensitization occurred
			SPT		RAST[1)]					
			n	(%)	n	Rhinitis	Lower airways[2)]	Total	n	
Baking industry (study I)	4 bakeries	153	12	(7.8)	5				5 out of 12 (42%)	α-amylase, fungal protease
	Baking	76	9	(11.8)		15	3	18		
	Packing	55	2	(3.6)		8	1	9		
	Office work	22	1	(4.5)		9	0	9		
	Flour mill	62	3	(4.8)	2					α-amylase
	Process work	56	3	(5.3)		13	5	18	1 out of 3	
	Office work	7	0			12	2	14		
	Rye crisp factory	150	4	(2.7)	0					cellulase,
	Manufacturing work	74	3	(4.1)		13	5	18	2 out of 4	glucose
	Packing	58	1	(1.7)		14	3	17		oxidase
	Office work	18	0			11	0	11		
Enzyme production (study II)	Biotechnical research laboratory	94	11	(11.7)	7	The plants combined, respiratory symptoms according exposure group[3)]:			The plants combined: 12 out of 21 (57%)	cellulase, xylanase, α-amylase (fungal), α-amylase (bacterial), phytase
	Research and laboratory work	79	10	(12.6)						
	Office work	15	1	(6.6)		rhinitis/ lower airways: Group 1: 27% / 10% Group 2: 2% / 4% Group 3: 1.7% / 0%				
	Biotechnical plant	79	10	(12.6)	7					
	Research and laboratory work	27	7	(25.9)						
	Enzyme manufacturing	25	1	(4)						
	Baker's yeast production	22	1	(4.5)						
	Office work	5	1	(20)						

Detergent industry (study III)	Detergent factory Manufacturing Packing Maintenance Laboratory work Storage work Cleaning Office work	76 17 7 5 6 4 1 36	9 5 2 2 0 0 0 0	(11.8) (29.4) (28.5) (40)	9	Process versus office personnel: Rhinitis 47% / 11% Lower airways 12% / 0%	9 out of 9	protease, lipase, cellulase
Animal feed industry (study IV)	4 animal feed factories Process work Office work	218 140 78	10 10 0	(4.6) (7.1)	3	Process versus office personnel: Rhinitis 16% / 8% Lower airways 4% / 4%	6 out of 10	cellulase, xylanase, phytase, glucoamylase, α-amylase, protease

[1] RAST performed if SPT was positive
[2] lower airways = cough or dyspnea
[3] exposure groups: 1 = often exposed; 2 = occasionally exposed; 3 = rarely or not exposed

Table 11. Association between atopy and sensitization to enzymes
(RR = rate ratio, 95% CI = 95% confidence interval)

Industry	Enzyme sensitization in atopic people (n/N)	%	Enzyme sensitization in nonatopic people (n/N)	%	RR	95% CI
Baking industry (process workers, n=318)	9/59	15	10/259	3.9	4.0	1.7–9.3
Enzyme production and biotechnical laboratories (groups 1+2: "often" + "occasionally " exposed, n=114)	10/33	30	9/81	11	2.7	1.2–6.1
Detergent factory (process workers, n=40)	3/14	21	6/26	23	0.9	0.3–3.2
Animal feed factories (process workers, n=140)	6/33	18	4/107	3.7	4.9	1.5–16.2
Total	28/139	20	29/473	6.1	3.3	2.0–5.3

As sensitization to enzymes occurred almost exclusively in the "process workers'' groups, the calculations were made for these groups.

5.6. Work-related symptoms

Work-related respiratory symptoms are summarized in Table 10.

Respiratory symptoms at work were frequently reported: in the baking industry by 9% of "office personnel" and 18% of "process personnel", in the detergent factory by 11% and 47%, and in the animal feed industry by 8% and 16%, respectively. In the biotechnical laboratories and the biotechnical plant, up to 27% of the workers in the group of the highest exposure reported symptoms. Most of the symptoms (>80%) were moderate rhinitic symptoms (rhinorrhea and/or stuffy nose). Symptoms indicating origin in the lower airways (recurrent cough, dyspnea) were reported by 5% of the bakers and 12% of the process workers in the detergent factory.

There was a significant association between sensitization to enzymes and work-related respiratory symptoms (Table 12).

Of the 59 people sensitized to enzymes in the workplaces studied, 34 reported work-related symptoms, 23 with rhinitis, 6 with recurrent

Table 12. Association between sensitization to enzymes and respiratory symptoms at work (RR = rate ratio, 95% CI = 95% confidence interval)

Industry	Symptoms in sensitized people (n/N)	%	Symptoms in nonsensitized people (n/N)	%	RR	95% CI
Baking industry (process workers, n=318)	8/19	46	45/299	15	2.8	1.5–5.1
Enzyme production and biotechnical laboratories (groups 1+2: "often" + "occasionally" exposed, n=114)	11/19	58	15/95	16	3.7	2.0–6.7
Detergent factory (process workers, n=40)	9/9	100	10/31	32	3.1	1.9–5.2
Animal feed factories (process workers, n=140)	6/10	60	24/130	18	3.3	1.7–6.1
Total	34/57	60	63/555	11	5.3	3.8–7.2

As sensitization to enzymes occurred almost exclusively in the "process workers" groups, the calculations were made for these groups.

cough, and 5 with dyspnea. Later, 18 of these 34 persons were also examined at FIOH. Enzyme-induced asthma was verified in 4 and rhinitis in 12 of them using specific challenge tests.

5.7. Specific challenge tests (study V)

Challenge tests with cellulase were performed on 11 patients. Symptoms were experienced by four patients at the lowest exposure level (30 mg of cellulase), by four patients at the second level (300 mg of cellulase), by two patients at the third level (3 g of cellulase) and by one patient at the fourth level (10 g of cellulase). Eight of the patients showed PEF drops of more than 15% in the challenge tests. Eight people reacted with rhinorrhea, two with pharyngeal symptoms and two with skin symptoms. There was an association with the level of sensitization, assessed by the RAST, and with the amount of enzyme needed to elicit the symptoms in the

challenges, three patients with high RAST values having responded to either 30 mg or 300 mg of cellulase, while the two RAST-negative patients responded to only 3 g or 10 g.

The monitoring of the air in the challenge chamber showed concentrations of cellulase ranging from 1 μg/m^3 to 1.3 mg/m^3 during the challenges.

5.8. Characterization of enzyme allergens

To characterize the allergens of the enzyme extracts used in the tests, we used sera of bakers and enzyme laboratory workers sensitized to fungal (*Aspergillus*) or bacterial (*Bacillus*) α-amylases, and *Trichoderma* cellulase-positive sera, in immunoblotting. Fungal amylase-positive IgE was shown to bind to a band of the fungal α-amylase with an MW of 50–60 kDa, but no binding occurred to bacterial (*Bacillus*) amylase. Cellulase-positive IgE bound to several antigens with MWs of 30–90 kDa (Figures 2–4) (Nordman et al 1993, Vanhanen et al 1994).

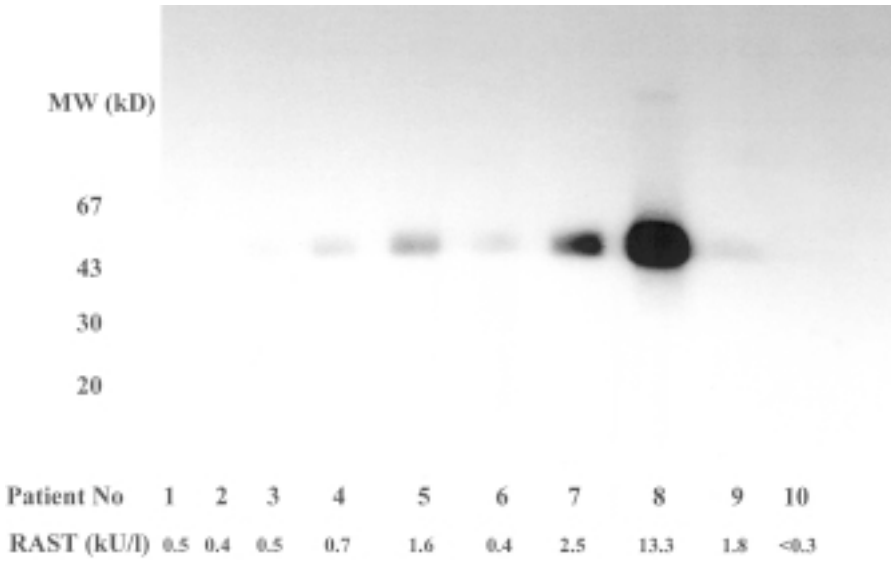

Figure 2. Binding of specific IgE antibodies to fungal α-amylase in immunoblotting. Numbers 1–9 are serums of amylase RAST-positive patients; number 10 is a control (RAST negative) serum.

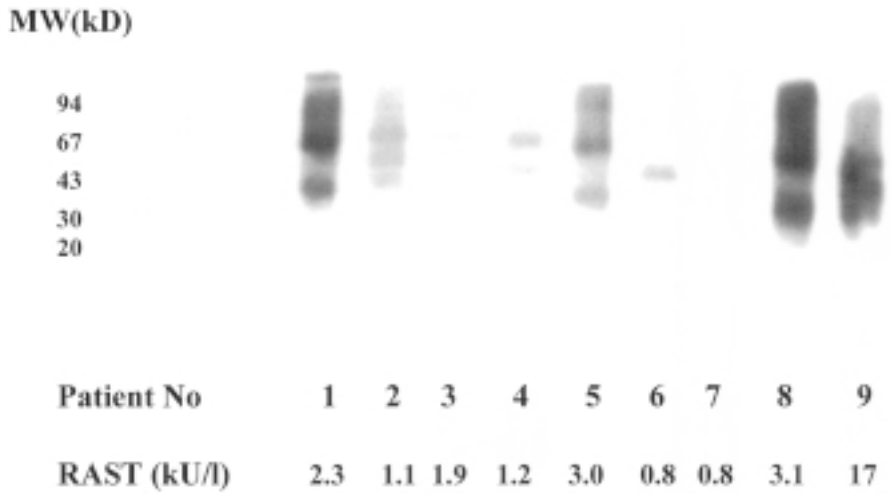

Figure 3. Binding of specific IgE antibodies to fungal cellulase in immunoblotting. Numbers 1–9 are cellulase RAST-positive sera.

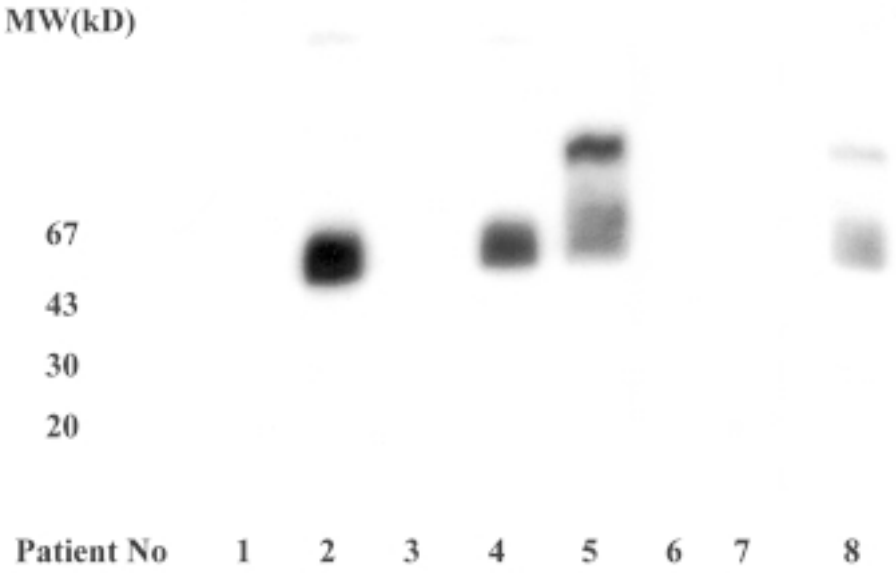

Figure 4 a.

Figure 4 b.

Figure 4 a–b. Binding of specific IgE antibodies to fungal (a) and bacterial (b) α-amylase in immunoblotting. Numbers 1, 3 and 6 are control (RAST negative) sera, numbers 2, 4, 5 and 8 are fungal amylase-positive sera, and 7 is a bacterial amylase-positive serum.

6. DISCUSSION

Large-scale use of microbial enzymes in industry started in the 1960s in the detergent industry and led to a wide allergy problem due to proteases in the late 1960s and early 1970s. Substantial improvements in industrial hygiene led to a clear decrease in the occurrence of the allergies. However, allergies were reported later for other applications, for example, in the pharmaceutical industry in the 1980s and, especially, in the baking industries since the mid-1980s. Powdered enzymes have commonly been used to improve dough in bakeries since the 1980s.

Our studies were initiated by the referral of cases of occupational asthma, rhinitis and dermatitis due to exposure to enzymes from the Finnish enzyme manufacturing industry in 1989–1990 (Tarvainen et al 1991).

It was learned that enzymes were used widely in Finnish industries, and new applications, using novel protein engineering technologies, were being developed constantly. Powdered enzyme preparations were in use in most bakeries, and employees were exposed to enzyme dust also in animal feed factories and the detergent industry, as well as in enzyme production. Consequently, these areas were chosen as the target for investigation. The detergent factory was intented to function primarily as a reference workplace for our studies in that the exposure to enzymes and the prevalence of allergies were expected to be low on the basis of the general assumption of the safety of encapsulated enzymes and the paucity of allergy reports from this industry since the 1970s.

6.1. Air concentration of dust and enzymes

6.1.1.Total dust

There was a great variation in the dust concentrations between the different workplaces and between the worksites of each workplace. In the bakeries, the total dust concentrations were generally less than 5 mg/m^3, which is the Finnish occupational exposure limit (OEL). As expected, the highest flour dust exposure was found in dough making, up to levels of 10 mg/m^3 in personal samples. Local exhaust ventilation was used in one bakery, lowering the exposure to levels of 3–5 mg/m^3 of total dust. The bakery results were in accordance with values published in different countries, especially during dough making, for which dust levels well over 10 mg/m^3 and even geometric

mean values on the order of 5–6 mg/m^3 are common (Tiikkainen et al 1996). Smith and Smith (1998) stated that "it is probably reasonable to assume that regular exposure to total inhalable dust from bread baking ingredients might be of the order of 5 mg/m^3 8 hour time-weighed average".

The total dust levels in the flour mill were of the same order as in the bakeries. The highest levels, up to 6.7 mg/m^3, were measured during the mixing operations.

The exposure was lowest in the rye crisp factory, with a mean value of 3.1 mg/m^3 for the personal samples and 0.8 mg/m^3 for the stationary samples. The low levels, when compared with the levels in the bakeries and the flour mill can be explained by the totally different "factory-like" processes and the automated handling of the flours.

The dust levels greatly varied also in the animal feed factories, the concentrations ranging from < 0.1 to 38 mg/m^3. There were no data available from animal feed industry elsewhere.

In the detergent factory the total dust exposure was clearly less than in the baking industry, ranging from <0.07 to 1.3 mg/m^3 in the personal samples. Few data on the total dust concentrations in other detergent factories were available for comparison: levels of 0.66–22.38 mg/m^3 from the late 1960s (McMurrain 1970) and 0.2–0.3 mg/m^3 from the 1970s (Juniper et al 1977).

There was a clear difference between the contents of the dusts of the detergent factory and those of the bakeries and animal feed factories. The dust in the detergent factory consisted mainly of inorganic ingredients of detergents. In the baking and animal feed industries the dust was mainly organic, originating from grains and flours.

6.1.2. Enzymes

We used catalytic methods to detect α-amylase and protease, and an immunologic method to measure cellulase, xylanase and protease.

In the bakeries, high α-amylase levels, up to 6.6 μg/m^3, were found in dough making, which is the dustiest job in general, and in which enzyme containing additives are handled. In other locations levels were generally lower, below 0.2 μg/m^3. The α-amylase levels were comparable to those reported by Jauhiainen et al (1993) in Finnish bakeries. The analysis of α-amylase was made with the same catalytic method. Since 1996, immunologic methods have been employed widely for α-amylase measurements in bakeries in Europe and Canada (Houba et al 1996, Houba et al 1997, Sander et al 1997, Burstyn et al 1998, Nieuwenhuijsen et al 1999, Elms et al 2001). Mean exposure

levels on the order of 10–30 ng/m^3 have been reported for dough making, with single peak values of up to 200–300 ng/m^3.

Xylanase concentrations of 2–200 ng/m^3 (mean 65 ng/m^3) were found, although xylanase was not a component in the dough improvers used in the bakeries. Wheat contains small amounts of inherent xylanolytic enzymes (Poutanen 1997), and the precence of these enzymes may explain the result. No previous reports were found on measurements of xylanase in bakeries.

The few measurements available from the enzyme manufacturing industry showed cellulase concentrations of 40–60 ng/m^3 during the weighing of samples in a laminar flow cabin. Descriptions of work practices in certain tasks, such as the mixing of powdered enzyme preparations, indicated that clearly higher enzyme levels than 60 ng/m^3 occurred at these sites. The measurements in the facilities of a subcontract plant revealed high values of 6–7 µg/m^3 for cellulase during packing, and extremely high values, up to 120 µg/m^3, in a spray-drying hall. The high exposure levels were also reflected in a survey in the subcontract plant, where seven cases of enzyme-induced disease, among a workforce of about 50 workers, were diagnosed (six cases of asthma and one case of rhinitis, three also having urticaria) (unpublished data).

In the animal feed factories, the high levels of protease (up to 360–2900 ng/m^3) and α-amylase (up to 200 ng/m^3) coincided with high total dust levels but not with the amount of added enzyme. No data are available for comparison on enzyme air concentrations in the animal feed industry elsewhere.

In the detergent factory, the total dust exposure was generally lower than in the aforementioned workplaces. In the more-automated laundry detergent production the protease levels were generally below 50 ng/m^3. The levels were surprisingly high at the mixing site during dishwashing detergent production (above 1000 ng/m^3); the analysis with the immunologic (Savinase) method using the same samples gave values of 56 and 62 ng/m^3. The exposure at this site had been recognized by the company, and hoods and respiratory protection had been arranged. In comparison, high levels of protease, from hundreds of nanograms to tens of micrograms per cubic meter, were reported in the detergent industry abroad in the 1970s and 1980s (Weill et al 1971, McMurrain 1970, Liss et al 1984, Schweigert et al 2000). In the 1990s, air measurements (with immunologic methods) revealed gradually diminishing exposures, generally below 15 ng/m^3. However, the long sampling time needed fails to recognize peak concentrations exceeding the average levels, due to, for example, systems failures. The role of peak exposures in the inducement of sensitization is not known.

When the α-amylase concentrations determined in the bakeries by the catalytic assay are compared with those measured with immunologic assays, the difference is on the order of about tenfold. Due to totally different measuring methods and standards, the results are not directly comparable. The difference not only reflects the differences in the fungal amylase concentrations in the bakeries, but it also indicates the inherent content of amylase in the flour, which is detected by the nonspecific catalytic method but not by the immunologic assay. In a study by Jauhiainen et al (1993), wheat flour contained an α-amylase concentration of 1.1 mg/g, whereas two commercial additives had an α-amylase content of 3.1 mg/g and 1.6 mg/g. Thus the amylase activity of the additives was only 1.5 to 3 times higher than that of the flour. Burdorf et al (1994), using a catalytic method to measure amylase, showed the total amylase content of flour dust to be 0.03% on the average. If it is assumed that the flour dust concentration in air is 2 mg/m^3, the amylase concentration would consequently be about 0.6 μg/m^3.

The parallel measurements of protease with the catalytic and the immunologic methods in the animal feed and detergent factories illustrated the specific and nonspecific nature of the methods. As expected, the immunologic method, detecting only a certain detergent protease, did not detect protease in the animal feed factories in spite of the high protease activities shown by the catalytic method. On the other hand, there was a correlation between the results of the two methods in the detergent factory. In the detergent factory the origin of the protease is the added enzymes, in contrast to the amylases and proteases with different origins in bakeries and animal feed factories.

As the catalytic method is based on enzymatic activity only, it is not specific as to the structure of the enzyme protein. Immunologic methods are more specific, as they are based on polyclonal or monoclonal antibodies towards certain purified enzyme proteins. Methods based on monoclonal antibodies seem to be even more specific than those based on polyclonal antibodies (Sander et al 1997, Elms et al 2001).

In animal feed factories, the origin of the protease activity remained unclear, but there are some possible explanations. Grain, especially in the stage of germination, has several enzymatic activities (Poutanen 1997). In addition, molds and mites, for example, have been shown to contain proteases and amylases as antigenic proteins (Robinson et al 1997, Robinson et al 1990, Lake et al 1991).

The immunologic assay for cellulase seemed to detect the added cellulase, as the highest levels (160–180 ng/m^3) were measured in the flour mill and crisp bread factory, where cellulase was used in additives. For comparison, there are no reported data on cellulase measurements in bakeries, or other industries, abroad.

The immunologic methods for enzyme detection have some advantages over the catalytic assays. First, the immunologic methods are specific as to the enzyme protein used in the additives, and this specificity is necessary in controlling the health hazards in industries using enzymes. Second, also inactive enzyme proteins (or parts of them) are detected. Such detection is important, as it is likely that also inactive enzyme proteins can act as allergens. One limitation of monoclonal assays is that the production of monoclonal antibodies is more costly and more time consuming than that of polyclonal antibodies. In addition, as the enzyme has probably several antigens and only one or few antigens are measured, one has to make sure that the main allergens are detected. The more specific the assay is, the more sensitivity is required to detect the minute amounts of the protein. A comparison of four immunologic methods used to assess α-amylase showed reasonably good agreement between the three methods using polyclonal antibodies, while a method with monoclonal antibodies showed a factor of three to six times higher values. It remained unclear why the monoclonal method gave higher values. A clear need for standardization was indicated (Lillienberg et al 2000). Internationally standardized and accepted sampling and assay methods would enable better development of the methods and the comparability of exposure levels, as was the practice when α-amylase was monitored in The Netherlands and the United Kingdom (Houba et al 1997, Burstyn et al 1998, Nieuwenhuijsen et al 1999). Standardized methods for measuring enzymes are also a prerequisite for the setting of future exposure limits.

In the development of methods and standard assays for measuring enzymes, difficulties arise from the fact that new enzyme profiles are developed constantly. For example, proteases used in the detergents, and amylases in baking, are being developed to tolerate different pH levels and temperatures better. It follows that the more specific a monitoring method is, the more vulnerable it will be in the future, as the structure of the enzyme may change and thus it may be left undetected by the antibody. Thus a more nonspecific catalytic assay, detecting all the amylolytic, or proteolytic, activity, might be more practicable in some instances.

6.2. Sensitization and allergy to enzymes

The prevalence of enzyme sensitization in the bakeries was 7.8% when all the employees were taken into account and 11.8% in the bakers' group. The prevalence coincides with those reported elsewhere in Europe, for example, 5–16% in the United Kingdom (Cullinan et al 1994, Smith & Smith 1998, Jeffrey et al 1999,

Nieuwenhuijsen et al 1999), 9% in The Netherlands (Houba et al 1996), 7.5% in Italy (DeZotti et al 1994), and 19% in Germany (Baur 1998a). The prevalence of flour sensitization in bakeries was 12%, which was on about the same order as elsewhere in Europe in the aforementioned studies (6%–24%). The employees sensitized to enzymes belonged to typical groups exposed to flour and enzymes, but a detailed exposure profile was not included in the original study design. Thus further exposure-response assessment was not possible. It was learned that the job tasks of many of the sensitized workers had varied over the years. The study demonstrated that exposure to powdered additives containing enzymes leads to sensitization in Finnish bakers. In the rye crisp factory, where exposure was less on the whole than in the bakeries, sensitization to enzymes was rare. Cellulase was shown to cause sensitization even at moderate exposure levels. There was a significant association between enzyme sensitization and work-related respiratory symptoms. However, co-sensitization with flours was common, and the origin of the symptoms was difficult to determine.

The animal feed industry is a new area of enzyme application. It was demonstrated that enzymes pose an allergy risk in this industry, too. The risk is smaller than, for example, in the baking industry, but, still, cases of occupational asthma due to enzymes have occurred. There was a correlation between sensitization to enzymes and work-related symptoms, and some of the sensitized workers reported symptoms when exposed to enzymes. One of the enzymes to which sensitization was shown was phytase, which has been developed solely for use in animal feeding. Previously, sensitization to phytase was found in Finnish enzyme production (study II). These were the first reports of allergy caused by this enzyme.

In the detergent factory, a surprisingly high prevalence (22%) of symptomatic sensitization was found among the process workers. In addition to established allergens in the industry, the bacterial proteases, sensitization to new enzymes such as lipase and cellulase was detected. This was the first publication on the allergenity of these enzymes in the detergent industry. Sensitization to bacterial α-amylase (Termamyl®) in the industry had been noted since the late 1980s (Sarlo et al 1997). In a later paper from the same multinational detergent company it was also reported that sensitization to lipase had been detected in the early 1990s (Peters et al 2001). In addition the present study showed that enzyme allergy is still possible, and it can even occur at a high rate, in the detergent industry, despite the use of encapsulated enzyme preparations that are principally considered nondusty and safe. This conclusion received support recently from the study of Cullinan et al (2000) in the United Kingdom. The prevalence of sensitization and clinical

allergy in these two studies was in clear contrast to the prevalences reported by large multinational companies (Cathcart et al 1997, Schweigert et al 2000, Peters et al 2001). Obviously there are large variations in industrial hygiene conditions between different plants. Small industries may find it economically difficult to meet all the standards of the industrial hygiene programs conducted in large companies, including periodic health checks and continuous monitoring of workplace air by immunoassays (Schweigert et al 2000, Nicholson et al 2001).

In the enzyme-producing industry, a high rate of sensitization, up to 25%, was noted in subgroups with high levels of exposure. The division into subgroups enabled exposure-response (sensitization and respiratory symptoms) calculations, and a statistically significant trend was found for both sensitization and symptoms. A unique feature was the high allergy risk of the laboratory personnel. It turned out that powdered enzymes had been handled rather carelessly, and, clearly, an awareness of the sensitizing properties of the cellulolytic enzymes was lacking.

In addition to the sensitization of the employees who handled enzymes themselves or worked in the vicinity of enzyme handling, some cases of sensitization were found among people not involved directly in production, for example, among the cleaning and maintenance personnel and also among office personnel with occasional exposure. Maintenance workers may be exposed to the highest peak concentrations of enzymes in the workplace. The practice of hiring personnel on a subcontract basis is becoming general, and this practice increases the probability of being exposed to enzymes. The experiences gained in the subcontract plant that spray-dried cellulase confirmed the importance of proper and sufficient information. The employees in the subcontract plant were clearly ignorant about the sensitizing properties of the substances they were handling.

6.3. Role of atopy in the sensitization to enzymes

Atopics, defined by SPT positivity to environmental allergens, were at greater risk than nonatopics with respect to sensitization to enzymes; the finding is in agreement with those of most reports on high MW sensitizers. However, also nonatopics had a marked risk, as 4–20% of the nonatopic “process workers” were sensitized.

Some data indicate that atopics are more suspectible to contract sensitization than nonatopics especially at low exposure levels in

bakeries (Houba et al 1996). Similar results have been reported by Heederik et al (1999). Atopic workers had a three-fold increased sensitization risk at low levels of rat urinary allergen exposure. The risk increased little with increasing exposure, whereas for nonatopic workers a steadily increasing risk was observed. On the basis of a reanalysis of data of a previous cross-sectional study on exposure-response relationships in bakery work, Heederik and Houba (2001) concluded that sensitization risk is twice as high for atopics than for nonatopics over the whole exposure range.

When the enzyme allergies emerged in the detergent industry in the late 1960s, the practice of excluding atopics from work with enzyme exposure was recommended (Newhouse et al 1970, Witmeur et al 1973, Juniper et al 1977). There were, however, other opinions stating that these measures may not be necessary when the alternative of adequate dust suppression is effective (Weill et al 1971), and for a long time it has been the policy of the large detergent manufacturers not to use atopy as a selection criteria during employee recruitment (Schweigert et al 2000, Nicholson et al 2001). This stand has also been generally agreed upon among researchers. Atopy is common in general population (Haahtela et al 1980). The exclusion of atopics is not justified; although they are at greater risk, not all atopics contract a disease, and also nonatopics become sensitized. Thus the focus should be in decreasing exposure (Nordman 1987, Nordman 1994b, Venables & Chan-Yeung 1997, Niven & Pickering 1999). On the other hand, people with current asthma, chronic rhinitis or eczema of the hands may be unsuitable for tasks with exposure to sensitizing organic dusts, be they atopic or not (De Zotti & Bovenzi 2000).

6.4. Diagnosing enzyme-induced asthma using specific bronchial provocation test

The challenge protocol used in this study appeared to be practical for powdered enzymes. The workplace conditions could be mimicked to a reasonable degree, as cellulase was commonly spray-dried and handled as powder at the workplace. The same method has been used for other dust provocations, such as flour and fungal α-amylase. The protocol also enables nasal and conjunctival symptoms to be assessed, and possibly also urticarial skin symptoms. However, the method is not suitable for liquid enzymes. Air monitoring of the enzyme was possible with the immonologic assay, but the method as such was time consuming, and only a few measurements were done.

The bronchial challenge test is generally accepted as the gold standard for diagnosing occupational asthma, especially when the

specific cause is searched for and when the suspected agent is new (Nordman 1994a, Chan-Yeung & Malo 1995, Cartier 1998, Cartier 1999). We performed the tests for these reasons. However, challenge tests for enzymes at a clinic are not always necessary (e.g., when sensitization is proved and the symptoms are typical, and PEFR monitoring is convincing for work-induced asthma).

6.5. Characterization of enzyme allergens

α-Amylases of different (fungal or bacterial) origin appeared to have different antigens, shown by the immunoblotting, as antigens of fungal α-amylase did not cross-react with antigens of bacterial α-amylase. This finding is important with respect to diagnostics, for example, when enzyme extracts for SPTs are chosen. It has a bearing also on the future use of immunologic methods for measuring enzyme concentrations in the air.

6.6. Validity issues

6.6.1. Study design and selection of study populations

All of the studies were cross-sectional, and the persons who had left the enterprises in question were not traced. Thus the populations were "survivors", as those with symptomatic allergy, at least asthmatics, had probably left the workplace. However, the occupational health units reported that few people had left the work due to asthma. The lower prevalence of atopy among the "process workers" group when compared with that of the "office" group indicates that some selection had occurred. Such selection could lead to an underestimation of the true prevalence of symptomatic allergy. The studies were planned in collaboration with the representatives of the personnel and occupational health units, and this step helped to maintain a high participation rate (>90%). Hence the population tested formed a good representation of the workplaces studied.

6.6.2. Validity of the methods

6.6.2.1. Assessment of sensitization

SPTs were performed with a routine and well-documented method. Enzyme test concentrations of 50 μg (protein)/ml (study I–II) or 100 μg/ml (studies III–V) were used. The shift to the stronger

concentration was prompted by experience indicating that the milder concentration caused rather small reactions in many workers for whom the RAST was positive. The stronger concentration gave clearer reactions but still reactions larger than those obtained with histamine were uncommon. In the literature the material and concentrations used in enzyme tests varies greatly. It is even common that the origin and the concentrations used are left undescribed. For fungal α-amylase, test concentrations of 1mg/ml (Baur 1998a), 5 mg/ml (Houba et al 1996), 10 mg/ml (Cullinan et al 1994, Nieuwenhuijsen et al 1999) and 50 mg/ml (Brisman & Belin 1991) have been reported. In comparison, the test concentration for workers in the bakeries (study I), 50 μg protein/ml, was low, and, accordingly, some cases of sensitization may have been missed. However, as the origin of the extracts differed, the concentration figures are not directly comparable. A large series of RAST tests were also made on bakers with negative SPTs, and they too were negative. For detergent bacterial proteases, Flindt used a concentration of 1 mg/ml (Flindt 1969); later varying concentrations between 0.01 mg/ml and 10 mg/ml have been described (Weill et al 1971, Pepys et al 1973, Belin & Norman 1977). Belin and Norman (1977) found that test concentrations greater than 1 mg/ml caused nonspecific irritant responses in unexposed control subjects. Their test preparations were standardized in terms of protein concentration.

6.6.2.2. Assessment of symptoms

Two sets of self-administered questionnaires were used: one in studies I–III and a new one in study IV. The first questionnaire was a modification of sets of questionnaires that had been used previously in several epidemiological studies concerning work-related allergies in Finland; it has not been validated. The second was based on the extensive Finnish Tuohilampi questionnaire (Susitaival & Husman 1996). The Tuohilampi questionnaire has been validated recently and the symptom-based questions were found to have high sensitivity and to be suitable for screening (Kilpeläinen et al 2001).

6.6.2.3. Assessment of exposure

At the time of the studies, in 1992–1997, industrial hygiene conditions were far better in enzyme production than what they had been earlier. In the detergent factory, a new factory building with new facilities had been built 10 years earlier, leading probably to a reduction in enzyme exposure. Likewise, a shift from dry enzyme preparation to liquid enzymes was occurring in the animal feed

industries. Consequently, the conditions seen and exposures measured may not reflect the conditions that caused the sensitization of workers in these industries. In the baking industry, however, the exposure to enzymes was probably at the same level as it had been since the start of enzyme use.

6.7. Prevention of allergies to enzymes

The high prevalences of enzyme sensitization and clinical allergy emphasize the need for preventive measures. Indeed, the major points of prevention were stressed already by Dr Flindt in 1969 (e.g. enclosure of processes, proper storing and cleaning methods, and sufficient personal protective equipment).

Guidelines for the safe use of enzymes have been published, for example, by enzyme producers (AMFEP 1994) and the detergent industry (Gilson et al 1976, Schweigert et al 2000). In Finland, a booklet on enzyme-containing flour additives, based partly on the experiences and results of study I, was published by the Ministry of Social and Health Affairs in 1997 (Aalto et al 1997).

Information is the fundamental basis of prevention. First, employers, employees and occupational health professionals must be aware of the properties of substances used at the workplace and the symptoms and diseases they can cause. Material safety data sheets should be available on substances containing enzymes, and the sensitizing properties of the substances should be indicated in them. Second, an industrial hygiene program to minimize exposure is mandatory. The experience from the detergent industry and the demonstrated exposure-response relationships prove that lowering exposure levels is beneficial. The change from powdered proteases to less dusty preparations proved to be beneficial in the detergent industry. This same change could be made in the baking industry, where the workers are clearly at risk. Paste and liquid baking additives are already available and a major Finnish bakery company has recently switched to using liquid enzymes in their processes. Technical solutions to lower flour and enzyme exposure have been developed. For example, with the introduction of local exhaust and a local air supply at a workstation, a reduction of up to 99.8% in the dust concentration in the breathing zone of workers was achieved (Heinonen et al 1996, Enbom & Säämänen 1998). Personal protective equipment can be used to supplement other measures for short periods in the dustiest phases (e.g., during dough making). The enclosure of processes and adequate ventilation help lower exposure. System failures of machinery cause high peak exposures and should

be minimized. Good work practice includes the careful handling of enzymes. Periodic health checks at occupational health units are widely used. The most important measure in this respect is to supply information and to inquire about work practices and work-related symptoms. Periodical SPTs are used in large companies as a biological monitoring tool for controlling industrial hygiene conditions (Schweigert et al 2000, Nicholson et al 2001). When sensitization is detected, an analysis can be made of work practices and the sufficiency of industrial hygiene measures. When the pitfalls and poor availability of air monitoring methods in many industries are taken into consideration, this method of biological monitoring is a practical tool that can be recommended.

The detergent industry has the longest history of monitoring enzyme concentrations in air. Air sampling can be used to determine the general exposure conditions and to ensure the effect of industrial hygiene improvements in the workplaces. However, due to the long sampling time, the measurements are still not capable of showing short duration peak exposures, which are not uncommon and may be important in eliciting sensitization (Peters et al 2001).

Besides the threshold limit value (TLV) for subtilisin (60 ng/m^3) set by the American Conference of Governmental Industrial Hygienists, there are no TLVs for other enzymes. The subtilisin TLV was based on few exposure data and experience has shown that it cannot serve as the NOAEL (no observable adverse effect level) for proteases or other enzymes. Consequently, the detergent industry has shifted to a guideline of 15 ng/m^3 for proteases, and used an even lower value for other enzymes (Schweigert et al 2000). According to reports by the industry, lowering exposure to low nanogram levels per cubic meter has almost put an end to new cases of clinical allergy (Schweigert et al 2000). Fungal α-amylase has been shown to cause sensitization in the low nanogram per cubic meter range (Houba et al 1997, Nieuwenhuijsen et al 1999), in comparison with sensitization to wheat flour in the microgram per cubic meter range (Houba et al 1998a, Houba et al 1998b). Data on animal experiments confirm the varying potency of different enzymes to elicit sensitization. A bacterial amylase, Termamyl®, was found to be three to ten times as potent as a protease, subtilisin (Sarlo et al 1997a, Sarlo et al 2000). It is probable that each enzyme is different as to its allergenic potency to humans. Moreover, multiple exposure to enzymes may modify the response, as indicated by animal experiments. Proteolytic enzymes in a mixture enhanced antibody responses to other enzymes in guinea pigs (Sarlo et al 1997b).

Setting a TLV requires that a valid method of monitoring the substance in the workplace air be available to users. The literature shows the need to keep at least protease and amylase levels lower

than 60 ng/m^3. Immunologic methods with very low detection limits are available, but there is still a need for the methods to be standardized. In addition, the constant development of new enzyme structures by the use of protein engineering creates a need for constant development of new antibodies and assays. Moreover, the technical problem of monitoring short peak exposures remains. In any case, a practical aim is the lowest possible level of enzymes in workplace air.

7. CONCLUSIONS

Exposure to enzyme proteins occurs in the research and production of enzymes, and in the manufacture of detergents, bakery products and animal feed where enzymes are used as additives or raw material.

In all the studied industries where exposure to enzymes occurred, sensitization was demonstrated for a significant proportion of the exposed workers. The prevalence of positive skin prick tests varied from 3% in the rye crisp factory to 22.5% in the detergent industry. An exposure-response relationship was established, sensitization being more common among highly exposed workers than among those in the lower exposure categories. The risk of sensitization to enzymes was elevated among the atopics. However, IgE-mediated allergy was noted also among the nonatopics.

Sensitization to enzymes increased the risk of work-related symptoms significantly in all the studied industries. In enzyme manufacture and biotechnical laboratories, work-related symptoms (rhinitis, recurrent cough, dyspnea) were significantly more common among workers exposed to enzymes than among the unexposed or rarely exposed persons, with a linear exposure-response trend.

In addition to previously well-known enzyme allergens, such as protease and α-amylase, the results emphasized the allergenic properties of other enzymes, such as cellulase, phytase and lipase. Allergy to cellulolytic enzymes was shown to be especially common in Finland. Phytase is used in animal feed and was shown for the first time to cause allergies.

The study showed that the structure of the enzyme protein is determined by its origin. Thus the antigens of fungal α-amylase did not cross-react with the antigens of bacterial α-amylase in immunoblotting. This phenomenon is important with respect to diagnostics and also has a bearing on the future use of immunologic methods for measuring enzyme concentrations in the air.

Development of internationally standardized assays for measuring air concentrations of enzymes is needed urgently. Experiences from this study show the need to use enzyme-specific methods to distinguish the added enzyme from the inherent ones in the product or material in question. On the other hand, more simple and inexpensive catalytic methods may be useful when, for example, interfering enzyme proteins do not exist or the total amount of detectable enzymes in air is to be determined.

Before enzyme allergy can be effectively prevented, sufficient information about the health hazards of enzyme use must be available

to employers, employees and occupational health personnel. Only then can the actions needed to minimize exposure be taken, for example, the application of local exhaust ventilation techniques and the use of proper work practices during the handling of enzyme products.

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